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THE PRINCIPAL INSECTS ATTACKING THE COCONUT  
PALM (PART II).

By CHARLES S. BANKS.

(From the Entomological Section of the Biological Laboratory, Bureau of Science.)

In Part I of this paper, insects which attack the trunk and the undeveloped leaves and flower clusters of the coconut were discussed. All the forms which have been described belong to the Coleoptera, but there are also certain species of Lepidoptera and Coccidæ which attack the coconut to a sufficient extent to warrant their being designated as injurious.<sup>1</sup>

Two forms of Lepidoptera are found upon the leaves of the coconut, one belonging to the Rhopalocera and the other to the Heterocera; the first is the coconut skipper, *Padraona chrysozona* Plötz, of the family Hesperiidæ, and the second, *Thosea cinereamarginata* Banks, of the Limacodidæ. While the nature of the damage done by the caterpillars of these two forms is very similar, the insects differ entirely from each other both in the larval and adult stage. Neither is likely to prove a very serious menace to the life of the tree. Each attacks the leaflets after they are practically full grown. A single caterpillar confines itself to a single leaflet until, with the exception of the midrib, it has entirely devoured it, whereupon it proceeds to another, and so on until the caterpillar has attained full growth. In the case of the coconut skipper, the caterpillar not infrequently eats a space from the blade of the leaflet at a point near its attachment to the main petiole, leaving the distal part untouched. (See Pl. I.)

<sup>1</sup>The bibliography of coconut insects, appended, includes all forms known to attack the tree, either here or in other countries, and is intended to be of further aid to those interested in the subject from an economic standpoint.

## THE COCONUT SKIPPER.

## LEPIDOPTERA.

## HESPERIIDÆ.

*Padraona chrysozona* Plötz.

During the months of September and October many of the leaflets of small coconut trees of from 6 to 15 feet in height are partially destroyed. Certain of these leaflets have their outer edges sewn together by means of a pure-white silk which is decidedly elastic, so that the leaf may be pulled slightly apart without tearing the fastening. Inside these folds the light, yellowish-green caterpillar, having a chitinous head, somewhat darker than the body and boldly marked with a very regular pattern, is encountered.

Toward the latter part of October the semiactive pupæ are found in these "cradles," partially covered and surrounded by a snow-white flocculent substance, which has a wax-like feel. This substance has very much the appearance of the wax secreted by certain species of Coccidæ and is exuded from the skin pores of the caterpillar toward the end of its larval stage. It serves as a protection for the pupa.

The coconut skipper, like nearly all Hesperiidæ, flies during the very early morning or the late afternoon and early evening hours, and hence it is very difficult to observe its egg-laying habits.

The eggs are found upon the under side of the leaflets of the coconut and but rarely more than one occurs upon a single leaflet. They hatch in from seven to eight days and the young caterpillar, after devouring all of the eggshell except that portion in contact with the leaf surface, at once proceeds to the edge of the leaf and begins to feed. This process consists in cutting out an oblique swath extending toward the midrib, of about the width of the insect's head. Frequently the caterpillar abandons a portion of the leaf, after having fed upon it for a short time, the result being that leaflets are encountered the margins of which are deeply notched, as shown by Plate II, fig. 1. Under normal conditions, and after the caterpillar has cut the leaflet to the midrib, it sews the margins together to form its nest, feeding upon the cut edge, either toward the apex or the base of the leaflet.

The neck of this caterpillar is much constricted, and therefore the head has considerable freedom of motion, but in a state of repose the normal dorso-ventral axis of the head is so inclined that it lies nearly in a plane with the longitudinal axis of the body, thus causing the mouth to be elevated and projected forward to form the extreme anterior point of the insect (Pl. II, fig. 3 A), which, in such larvæ as those of *Attacus atlas* Linn., and *Thosea cinereamarginata* Banks, is formed by the front of the face or the occiput. The caterpillar of the latter has the head deflected beneath the body.

## DESCRIPTION AND LIFE HISTORY.

*Egg* (Pl. II, fig. 2 and 2 A).—Diameter 1.65 millimeters, height 0.85 millimeter; of a rather flat, oblate-spheroidal shape, yellow-glabrous when first laid, with crimson, subcentral ring and central spot covering the micropyle, and developing after two days. The under surface nearly flat, glabrous; upper surface minutely punctured. It adheres strongly to the leaf surface.

The eggs of *Padraona chrysozona* Plötz are always laid singly upon the leaf, thus differing from those of *Erionota thrax* Fabr., which may be found in groups of from 8 to 15.

The period of incubation is from seven to eight days.

*Larva* (Pl. II, fig. 3).—Length 3.5 millimeters, width of head 1.2 millimeters; upon emerging from the egg. At this stage the larva is of a pale, greenish-yellow, with a black head, the size of which appears somewhat disproportionate to that of the body. A very fine, light-grey, sparse pile covers the body, especially the posterior segment.

The full-grown larva measures 45 millimeters in length and 4.5 millimeters in width, the head being 3.75 millimeters in diameter. It is of a pale, ocher-green, semi-transparent, permitting the viscera, especially the heart and the malpighian and urinary glands, to be seen readily through the skin. The head, which is about one-tenth the length of the entire body, is biscuit shaped or of a very flat, oblate-spheroidal. The surface is strongly and coarsely punctured. The ecdysical sutures are strongly marked by narrow sulci. It is of a glabrous, tawny, flesh-color. A dark-brown line extends from the base of each mandible around the side of the head to the occiput, where it is deflected forward, following the ecdysical suture and being again deflected toward the side of the head, ending in a sharp point, the lines of each side thus forming a Y on the median, dorsal aspect of the head. The ocelli, which lie in the beginning of the dark line posterior to the mandibles, are 6 in number on each side and of a dark-brown. The mouth parts are dark-brown and glabrous and are surrounded by a rather coarse, porrect pile. The anal segment is glabrous and its posterior margin is strongly rounded, with numerous, white, curved setæ projecting from it posteriorly. The legs are light yellowish-buff with many white setæ on their lower surfaces. The abdominal feet, of which there are 10, are strongly pubescent. The spiracles, which are functional on the 1st and 4th to the last body segments, are of a light-yellow.

*Pupa* (Pl. II, fig. 4 and 4 A).—Length 25 millimeters, width 4.5 millimeters. The pupa is of a glabrous, dark-brown, but is frequently so covered with a white, flocculent substance that its true color is not apparent. It is strongly seto-pilose, especially on the anterior dorsal part of the head and thorax and on the abdominal segments. The setæ upon the thorax project anteriorly, those upon the abdomen posteriorly. Very dark-brown rings extend around the apical margins of the 4th, 5th, and 6th segments. The proboscis extends to the apex of the 6th, its apical fourth being force free and transversely rugose.

A very remarkable feature of this pupa is the form assumed by the prothoracic spiracles. They are completely protected by a reniform patch of dense setæ and are located one on each side of the posterior dorsal margin of the pronotum. See Pl. II fig. 4 B.

*Imago*, male (Pl. III, fig. 1).—Length of body 15–16 millimeters, length of fore-wing, 15.5–17.5 millimeters. Ground color, bright, yellow-ocher, with the following dark-brown markings or suffusions: The veins and a more or less obliterated longitudinal patch along the posterior part of vein VII<sub>1</sub>, and VII<sub>2</sub>, from the base for one-half its length, in some specimens suffused with yellow-ocher; a similar, somewhat wider patch from the end of the cell to a point its own length from the outer margin. In some specimens this patch is confluent with the basal

one, forming an irregular, oblique band across the disc of the wing from the base to near the apex. Exterior to this wide patch, a yellow-ocher, irregularly rhomboidal spot, divided by the dark-brown vein  $V_1$ . A broad, marginal, dark-brown band from the costa to the posterior angle of wing, its inner margin scalloped and its area crossed by ends of veins which show yellow-ocher; a dark-brown suffusion at the base of and posterior to vein IX. Cilia, black, suffused with yellow-ocher toward the apex and yellow-ocher toward the posterior angle. The inner margin clothed with long yellow-ochraceous hairs. The hind wings with ground color dark violet-brown at the base, with iridescent, subcostal scales. Margin, dark-brown; cilia, yellow-ocher. A longitudinal, yellow-ochraceous patch in the cell, from which spring long hairs of the same color. A broad, yellow-ochraceous, irregular patch extends across the wing from the outer third of vein III to the middle of vein IX, which it follows to the base and margin of the wing respectively. Posterior to the cell patch is another one of long, yellow-ochraceous hairs extending to the oblique band. A similar patch extends along the inner submarginal area its own width from the inner margin of the wing.

The under surfaces of wings yellow-ocher; veins, marginal hair line, base and inner margin to vein IX and submarginal series of suffused spots on fore wing, dark-brown. On the hind wing the superior, oblique, yellow-ochraceous patch has its margin indicated below by series of faint brown spots or irrorationes. Antennæ brown, with dark yellow-ochraceous tips and lighter scales beneath. Palpi, sternum, venter, legs and apical margins of abdominal segments, yellow-ocher. Apex of abdomen dark violet-brown with yellow-ocher irrorationes. Removal of the head in both sexes reveals a patch of very broad, pearl-colored scales dorsally at the neck. Under normal conditions these scales are completely hidden by the hairs upon the head and thorax.

Female (Pl. III, fig. 2).—Length of body 17.5–20 millimeters, length of wing 18.5–21 millimeters. Ground color, dark violet-brown, especially on the veins of the wings; iridescent in certain lights.

The bases of the fore and hind wings, the head, thorax, the abdomen dorsally and the entire lower surface suffused with greenish-buff. Fore-wings above, with an irregularly, sub-rhomboidal spot in the end of the cell, 2 oblique, parallel lines between this and the costa, 3 parallel patches beyond them, near the costa, an oblique, interrupted band, from the outer third vein of  $V_1$  to the middle of inner margin, buff, irrorationed with brownish scales. Beyond the oblique band an irregular patch between veins III<sub>1</sub> and  $V_2$  interrupted by vein  $V_1$ . Inner margin with greenish-buff hairs for two-thirds its length. Cilia brown and greenish-buff.

Hind-wings as described above, with an ill-defined, interrupted, oblique, buff band from the middle of vein V to the middle of vein IX along which it extends as in the male. Cilia, buff. Inner submarginal area with long, greenish-buff hairs.

Beneath; wings brown, entirely suffused with greenish buff; the markings, which are buff above, are very pale-buff below and both wings have distinct, dark-brown veins and marginal lines.

Antennæ dark-brown above, and buff beneath to the base of club; bases of antennæ, black. Palpi, greenish-buff; apical segment, dark-ocher. Thorax and legs covered with dark, greenish-buff scales and hairs; tarsi, with reddish-brown spines beneath. Abdomen with 5 distinct, transverse, black lines and 3 indistinct apical orange ones dorsally. Anal region with orange scales.

Semper<sup>2</sup> says of this species: "This beautiful species is very easily recognized by the 3 yellow rings of the abdomen. It belongs next to *augiades* Felder and *palmarum* Moore, in which the males also lack the sexual sign [discal patch], upon the forewings, which appears in the female of *augias* L., and *bambusæ* Moore.

<sup>2</sup> Semper: *Reisen auf den Philippinen* (1892), 2 No. 5, 301 et seq.

Upon this ground, I believe, Staudinger's opinion that *bambusa* is a variety of *augiades* can never be right according to my way of thinking."

"The female of *chrysozona* varies greatly in the width of the dark markings; I have some which are as dark upon the upper side of the forewings as Moore's *palmarum*, others again have, with exception of the border, only fine, black stripes along the veins and a faint, dark shadow on the end of the cell. The ground color is darker than in *palmarum*, and the same as in *augiades*. The female on the upper side is exactly the same as the drawing of *palmarum*, but differs on the underside in that the light bands are nearly as clearly marked as they are above. The ground color is greenish-gray-brown."

This species is also found upon the betel palm (*Areca catechu* L.); in fact, Semper indicates in his note concerning this insect that the larva is only encountered upon that tree. My observations disprove this statement; indeed, it rarely is seen on any palm other than the coconut.

*Preventives and remedies.*—This insect is never found in sufficient numbers to justify the fear that it may become a serious menace to coconut culture, but as its feeding upon the leaves of small trees may have a tendency to debilitate them, its larvæ should be destroyed whenever they are encountered.

*Parasites.*—This insect is probably, to a great extent, held in check by two small Hymenopterous parasites, *Chalcis obscurata* Walk., and an unidentified Braconid, both of which attack the larva, laying their eggs within its body, their young feeding upon its fats and body fluids. The larvæ of the former parasite, of which there may be as many as 10, pupate within the pupa of the coconut skipper, which they kill, emerging therefrom in from five to six days thereafter (Pl. IV, fig. 1); those of the Braconid leave their host when they are full grown and, like all true Braconidæ, they spin pure-white cocoons in the vicinity of the now dead and shriveled caterpillar. After spinning their cocoons the insects emerge in about 4 days. (Pl. IV, fig. 2.)

#### DESCRIPTION OF PARASITE.

Walker's description of the Chalcid is as follows:

*Chalcis obscurata* Walk., *Proc. Ent. Soc. Lond.* (1874) 399.

*Male.*—Body, antennæ and legs black, with the usual structure. Body convex. Head and thorax scabrous, dull. Antennæ stout, nearly filiform. Prothorax about four times as broad as long. Sutures of the parapsides, distinct. Abdomen smooth, shining, subsessile, with cinereous tomentum toward the tip. Femora yellow at the tips, hind femora minutely denticulated beneath. Tibiæ yellow, striped beneath with black; hind tibiæ black at the base. Tarsi yellow, tips black. Wings cinereous; squamulæ yellow; veins black; ulna about half as long as the humerus.

*Hab.*: Hiogo (George Lewis), Philippines (Banks).

This is the first record of this species of the Chalcididæ as from the Philippines.

## THE COCONUT-SLUG-CATERPILLAR.

## LEPIDOPTERA.

## LIMACODIDÆ.

*Thosea cinereamarginata* Banks.*Thosea cinereamarginata* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 229.

The slug-caterpillar is easily distinguished from other Lepidopterous larvæ by the form of its body and its mode of locomotion, which is more like that of slugs or snails than of insect larvæ. Several species are known in the Philippines, many of them feeding upon palms. In Manila, this caterpillar is quite common and is usually found feeding either upon the upper or the lower surface of the leaves of the coconut. It presents a rather forbidding aspect, due to its being well armed with a double series of spinous tubercles placed upon either side, but, as a matter of fact, unlike most Limacodidæ, it possesses no poisonous properties. I have handled the larvæ freely without experiencing any discomfort.

The damage which this insect does to the coconut leaves is about equal to that of *Padraona chrysozona* Plötz.

*Egg*.—Diameter 1.5 millimeters, height 0.95 millimeters; of a flat, oblate-spheroidal shape and with minute reticulations upon the surface, pale-ochraceous. The larva escapes through a slit which divides the shell across its face, and the latter is not eaten as in the case of *Padraona chrysozona* Plötz. The period of incubation is from 5-7 days.

*Larva* (Pl. V, fig. 1).—When full grown, length, 23.75 millimeters, width, 14.25 millimeters including the tubercles. It is pale-green above and pale greenish-yellow at the sides, being almost pure-white beneath, and with the following markings: a median, light-purple or heliotrope band with symmetrical scalloped margin, the scallops expanding upon the respective segments. The margin of this band is darker purple and shades into the green of the dorsum. The band is developed into more or less of a patch upon the fourth and seventh segments, where the colors are darker. External to this band, on either side, is a series of 9 horizontally-projecting, spiniferous tubercles, those upon the second, fourth, sixth, eighth, and eleventh being twice or slightly more than twice as long as the remaining ones. Below these, ventrally on either side, is a series of 8 light-purple spots, one on each segment from the third posteriorly, and below these another series of 8 spots beginning upon the 4th segment. At the latero-ventral angle, a series of 11 horizontally projecting spiniferous tubercles, one of which projects anteriorly and another posteriorly on either side. The spines of these tubercles interlace and are yellowish-green at their bases and purple or black at their tips. Many of the spines have a white hair at their tips, and the shorter ones at the bases of all tubercles have somewhat inflated tips. The head is yellow-green and when the caterpillar is not feeding, is retracted within the 1st thoracic segment. The length of the larval stage varies from 21-25 days.

*Pupa* (Pl. V., figs. 2 and 2 A).—Length 8.5 millimeters, width 5.5 millimeters. The color is a light yellow, with ochraceous bands on the posterior margins of the dorsal, abdominal segments; the wing pads are livid flesh-color and the eyes are dark-gray.

*Cocoon*.—Length, 8.75 millimeters; dark-brown, cocciform or oblate ovoid, composed of finely comminuted leaf fiber held together with silk. The interior is white and silk lined. The pupal stage lasts about 22 days. Time of flight, the month of January.

*Adult*.—A description of the adult male and female of this species occurs in the *Phil. Journ. Sci.* (1906) 1, No. 3 p. 229. It is quite closely related to *Thosea minima* Semper, from which, according to Semper's description, it differs chiefly in having the prominent antemedial oblique sinuate band extending from near the cell spot to the middle of the inner margin.

*Preventives and remedies*.—The same methods of treatment apply to this species as to the Coconut skipper, though, as in the case of the other, there is no possibility of its ever becoming a serious pest.

#### SCALE INSECTS.

With exception of a few species from which useful or commercial products are obtained, such as the cochineal insect, *Llaveia cacti* Linn., and the lac insect, *Tachardia lacca* Kerr, practically all known species of scale insects are detrimental to man's agricultural interests. In some parts of the world they do more damage to crops and trees than is due to the effects of all the other insects of the region. While, in the Philippines, this is not so strikingly true in the case of the coconut palm, still the damage done to this tree by species of the family Coccidae is very considerable. It is rare to find a coconut which does not, by its yellow or brown leaves, indicate the ravages of these pests. Scale insects differ so greatly from ordinary insects that they may easily escape detection. As a rule, the commoner forms appear merely as rusty-brown or yellowish patches upon the surfaces of the leaves, or on the bark of the stems or trunk of the plant.

*Characters*.—In all species the body of the adult female is either covered with a scale formed of a waxy secretion in which the exuviae of the earlier stages are compacted or else the body of the insect itself assumes a form which suggests a scale or tubercle upon the host plant. The males of all species are winged, but on account of their very minute size and pale colors escape notice unless they are bred upon the food plant under glass, in which case they may be captured upon emerging as adults. The newly hatched young of both sexes are, of course, much smaller than the adults of either sex and it is almost impossible to see them with the naked eye.

Upon hatching, the young, coming from beneath the parent scale, scatter upon the leaf surface in quest of a favorable place to settle. Shortly after their first meal, which is obtained by inserting their probosces into the succulent part of the leaf or twig, the insects shed their skins, but during the period of feeding there will have exuded from certain body pores a pale, wax-like secretion which, adhering to the first exuviae, after a brief period assumes the form of a scale-like covering.

In the first molt the females usually shed their legs and sometimes their antennæ, so that a female nymph or adult appears as a mere sack, attached to the plant by the proboscis. The female, in those species having a distinct scale, remains under this covering throughout life, while the male, after a succession of molts, comes forth with legs and wings well developed.

In view of the great difference in appearance between the male and the female, it is necessary to consider their respective characteristics separately for purposes of classification, it being impossible to identify two given specimens of different sex as belonging to the same species unless they are found in close association or are bred from a given lot. In view of the relative scarcity of males in most genera of Coccidæ, the characters found in the female form the chief basis upon which their determination is made.

In the Philippines, so far as is known, seven species of Coccidæ are found upon the coconut. Of these, *Aspidiotus destructor* Sign. is by far the most abundant and destructive; next in abundance is *Chrysomphalus prosimus* Banks, a species which has usually been encountered in great numbers on all trees examined both in Manila and in the provinces. The order of abundance of the remaining species is that of the following notes:

#### THE TRANSPARENT SCALE.

##### *Aspidiotus destructor* Sign.

This extremely prolific scale is found on the coconut palm in all localities in the Archipelago where investigations have been conducted. It is extremely injurious to the trees, causing their leaves to assume a characteristic yellow color, which is easily noted from a distance. Where it is encountered, the under surfaces of the leaflets are covered with thousands of small, rough, circular patches, which are almost transparent and so thin that the insect and her eggs can be seen beneath. When the leaflet is pulled longitudinally or when it wilts, the scales become striated owing to the tension on the edges which are attached to the leaflet. Plate VI, fig. 1, shows adults and young scales upon a leaflet. It will be noted that the latter have fixed themselves to the longitudinal veins and therefore are arranged in very regular rows. Fig. 1 A shows young insects which have emerged from beneath the scale of the parent; female scales from which the occupants have been eaten by a tiny predaceous beetle of the family Coccinellidæ, are also present. Fig. 2 shows a coconut leaflet attacked by a form of disease which causes spots, very similar to those resulting from the attacks of *Aspidiotus destructor* Sign., to appear on the upper surface. In case of doubt as to the origin of the spots, certainty is reached by examining the underside of the leaflet, where, if it is attacked by scale insects, the latter will be found just at the point of discoloration of the leaflet.



## DESCRIPTION.

*Egg* (Pl. VII, fig. 4).—Length 0.2 millimeter, width 0.1 millimeter, regularly, bluntly oval, one side more convex, very pale lemon yellow, smooth, laid in two or three more or less regular concentric rows around the parent within the scale. This regular distribution necessitates a nearly complete rotation of the female around the point of insertion of the proboscis. This is accomplished by an undulating motion of the body and may be observed by placing the live insect upon a piece of glass, under the microscope.

*Larva* (Pl. VII, fig. 8).—Immediately after hatching the length is 0.25 millimeter, the width 0.15 millimeter. Pale yellow, slightly lighter in color than egg. Eyes dark red; antennae 5 jointed, slightly setose, last joint 3 times as long as the first 4; transversely, microscopically striate, biapical as shown by Plate VII, fig. 5, with a single seta from each apex; mouth two-fifths of distance from frontal to anal margin. Anal margin dentate, giving indication in both sexes of existence of pygidial lobes. These disappear in the male upon the second molt. Legs moderately long; femora somewhat stout. Tarsi single jointed with 2 knobbed spines on the dorsal margin. Proboscis about as long as the body. Four minute hairs project from the frontal and 2 from the anal margin of body, the anal being 4 times the length of the longest frontal.

*Male puparium*.—Oblong-oval, pale, translucent, larval exuviae at center slightly darker, yellowish. Plate VII, fig. 3, shows the male puparium.

*Female puparium* (Pl. VII, fig. 2).—Differs from male in being more nearly circular. Color as in male. Larval exuviae at or slightly removed from the center. Darker than the scale itself, yellowish.

Signoret's description\* is very meager. It is as follows:

"The scale is round, of a transparent white, with the exuviae at the center, and of a yellowish transparent white.

The female is yellow, round; the extremity with six lobes, of which the two median are shortest; the pygidium with four groups of wax glands of eight to ten orifices in each [group] agglomeration."

He says further concerning the insect: "This species appears to cause great damage to coconut groves in the Island of Réunion, where they are menaced with complete destruction. The scale is also found on palms and dates. We have found it likewise on *Goyavius psidium* [*Psidium guajava*] which we received in the same package."

It will be seen that Signoret's description is not sufficiently detailed to differentiate this scale from other very similar ones, as he makes no mention of the squames, their number, and arrangement, which, for example, is a very important point in distinguishing *Aspidiotus destructor* Sign., from *A. lataniae* Sign. The following description has been prepared from fresh material:

*Adult female* (Pl. VII, fig. 2).—Length 0.80–0.90 millimeter, width 0.65–0.75 millimeter, bright pale-yellow, broadly oval, nearly circular, narrowed posteriorly, with slight emargination at the base of the pygidium, which is only slightly paler; posterior margin, whitish creamy, due to waxy secretions; two submedian white spots on each side of the genital aperture show the position of the circumgenital glands, the posterior of which have 4 to 5 apertures, the anterior 7 to 9. Anterior margin of the body regularly rounded, abdominal segmentation laterally distinct. Antennae, small, oval knobs with inwardly curving bristles at the apex, situated one-third of the distance from the frontal margin to the rostrum. Between the

\* *Ann. Soc. Ent. de France* (1869), (4) 9, 120; Plate XII, figs. 8 and 8a. Translation.

antennae on the ventral surface are from 5 to 10 minute spinous tubercles. Parastigmatic glands not present. Stigmata subcylindrical. Pygidium (Pl. VII, fig. 6) with 6 lobes, median pair shorter than the next, weakly tricuspid, light-brown, next pair bicuspid, slightly paler, exterior pair of the same color, bicuspid, of the same length as the median. Chitinous portions of all lobes run anteriorly for some distance into the pygidial area, the surface of which is closely, longitudinally striate. Squames as follows for each side: 2, apically fimbriated, between the median lobes, similar ones, but more slender, between 1st and 2d lobes, 3 stouter between 2d and 3d lobes, with fimbriation somewhat externally laterad of apex; a series of 9 broad, laterally fimbriated ones beyond the 3d lobe and extending one-third the distance from the latter to the base of pygidial margin. These squames decrease in length and increase in width from the third lobe and the number and length of their fimbriations decrease so that the last one bears but 1 prominent spine, the remainder being reduced to sharp serrations of its latero-apical margin. Setae placed as follows: One pair at the external base of each median lobe nearly twice the length of latter, 1 pair between the second lobe and its 1st external squame, 1 pair ventrad to external lobe, 1 very short; ventrad to the 4th of the exterior 9 squames, 1 ventrad to the last squame, a small setose tubercle near the ventral margin, two-thirds of the distance from the last squame to the base of the pygidium. Four groups of circumgenital glands, the posterior pair with 4 to 6 orifices, the anterior with 7 to 12. Tubular spinnerets filiform; their heads chitinous; tube obconical, chitinous; their tongues one-third the diameter of their heads and equal to them in length. Numerous trumpet-shaped or subcylindrical ducts toward the apex and having orifices on the margin, as do tubular spinnerets. Anal opening about halfway from genital orifice to posterior apex. For details of structure see Plate VII, fig. 6.

*Adult male* (Pl. VII, fig. 1).—Pale-yellow, with darker, pinkish-yellow, transverse apodema. Head about one-tenth the length of entire body, including genital sheath. Ocelli very dark red; upper pair slightly extra-marginal, lower pair posterior and contiguous on median line, their diameter one-fourth greater than upper ocelli. True eyes posterior to and their own diameter distant from the upper ocelli, submarginal. Antennae composed of 10 joints, of which the first 2 are subglobular, 3 to 5 subequal in length and about 3 times the length of second, all segments sparsely setose. Tenth segment attenuated, terminating in a clubbed hair, surrounded by 3 other curved hairs of equal length. This is shown by Plate VII, fig. 7. Legs moderately long, posterior femora slightly stouter than the others. The single jointed tarsi, two-thirds the length of tibiae, slender, subconical and moderately covered with spinous hairs. Tibiae subequal to femora, with a very few hairs each. Claws or ungues one-fourth the length of tarsi, their digitules two-thirds their length. Tarsal digitule as long as ungues. Genital sheath long and tapering to a sharp point; two-sevenths of the length of the rest of the body. Wings iridescent, hyaline, obovate; veins of about equal length and subparallel to the respective margins. Haltere has the first joint swollen to about one-third its length, just before the apex. Second joint of length equal to the first; spinous and hooked at extremity. Length of wing 0.63 millimeter. This insect so nearly resembles *Aspidiotus lataniae* Sign. that it is very difficult to separate the two species. The chief points of difference in the female are the number of squames external to the outer lobe, the number of orifices in the circumgenital glands, and the relative length of the median and second lobes of the pygidium, the median in *A. destructor* Sign., being shorter than the 2d pair, while in *A. lataniae* Sign., they are of equal length and more markedly tricuspid.

*Aspidiotus destructor* Sign., is by far the most pernicious of the scales which attack the coconut in the Philippines. It most frequently occurs on young trees having from one to five years' growth, in many cases completely covering the under surfaces of all the leaves, giving them a characteristic yellow tinge. It is certain that it is attacked by a Hymenopterous parasite, as female puparia have been found showing the exit holes of the adult parasite, but as yet the latter itself has not been discovered. A small Coccinellid beetle, *Scymnus* sp., is a voracious feeder upon the transparent scale, the adults as well as the larvæ of this species frequently being encountered in considerable numbers upon coconut leaves which are covered with the scales. A description of this insect follows:

*Scymnus* sp.

*Larva*.—Length 1.75 millimeters, width 1.01 millimeters, exclusive of the pure-white waxy tufts which project from the front, sides, and posterior margins of the body as shown by Plate VIII, fig. 2. The larva of this beetle when once known can easily be distinguished from all others which might be found among scale insects in this region. It is extremely active, running from place to place and greedily gnawing open the delicate scale in order to obtain the insect which lies beneath. The body is pale yellow with a greyish tinge.

*Pupa*.—Length 1.25 millimeters, of a light ochre-yellow. This insect pupates within the larval skin, as do many species of the family. In this case the skin splits along the median dorsal line, exposing the pupa.

*Adult* (Pl. VIII, fig. 1).—Length 1.35 millimeters, width 1 millimeter, of a dark brown, almost black, with a light-brown discal spot on each elytron. In some specimens this spot is sharply, in others ill, defined. The entire body covered with a fine, white, pubescence. Palpi, apices of femora, tibiae and tarsi, brownish ochre. (See Pl. VIII, figs. 3 and 4 for antenna and palpi.)

*Habits*. This beetle, the adult as well as the larva, feeds on many species of Coccidæ but has been found in greatest abundance in colonies of *Aspidiotus destructor* Sign.

*Chrysomphalus proppius* Banks.

*Chrysomphalus proppius* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 230. (Pls. I and II.)

This scale bears a general resemblance to *C. aonidium* Linn., but its color and size, together with its apparent predilection for the coconut palm, upon which it is always found, make its identification as a distinct species a matter of some doubt. The scales crowd themselves upon both surfaces of the leaves of neglected or deformed trees and frequently as many as 4 or 5 are found overlapping each other. In Manila they breed with great rapidity and soon cover the leaflets and even the petioles. The same species has been encountered in great numbers upon the betel palm (*Areca catechu* L.) at San Miguel de Mayumo, Province of Bulacan. It may be distinguished from other scale insects which might be found upon the coconut by the decided, shining red-orange color of the pellicles. The male scales are infrequently met with in comparison with those of the female. (See Pl. X.)

*Parlatoria greeni* Banks.

*Parlatoria greeni* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 231. (Pl. III, figs. 1 to 6.)

This delicate, though prolific, scale is frequently seen in Manila upon young, badly cared for coconut trees. It is found upon the upper surfaces of the leaves and because of its flat shape and gray color is difficult to detect. While not as serious a menace as either of the foregoing species, it merits attention because of the possibility of its great increase if it is left unchecked.

*Chionaspis candida* Banks.

*Chionaspis candida* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 232. (Pl. IV, figs. 1 to 5.)

Frequently coconut trees are found the partially opened leaflets of which are covered with small, pure-white spots, due to the scales of another species of insect differing totally as to color and form from the foregoing. This scale multiplies rapidly upon either surface of the leaf, usually in the protected parts. As a rule, the female puparium occurs near to a group of male puparia or else with a group of the young scales in their first or second molt (Pl. IX). This insect is not as nomadic as *Aspidiotus destructor* Sign., therefore its opportunity for debilitating the tree is not as great and the danger from it is not to be feared in the same degree as from *A. destructor* Sign.

*Lepidosaphes mcgregori* Banks.

*Lepidosaphes mcgregori* Banks, *Phil. Jour. Sci.* (1906), 1, No. 3, p. 233. (Pls. V and VI.)

This scale is comparatively rare. It occurs upon both sides of the leaves of the coconut, especially on old ones, but seems to prefer that part of the upper surface that is near to the midrib. It is always encountered singly and the puparia are seldom distorted as in the case with *Chionaspis candida* Banks. Although rare, it may at any time and under favorable conditions propagate to the extent of being injurious. The most noteworthy features which distinguish it are the pair of white, waxy, horn-like projections on the front of the first pellicle, the light color of the female puparium and the regularity of its transverse striæ.

*Lepidosaphes unicolor* Banks.

*Lepidosaphes unicolor* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 234. (Pl. VII, figs. 1 to 7.)

This species is of nearly the same shape and size as the preceding, being only distinguished externally by the narrower, interior margin, the color of the puparium and the absence of the waxy horns in old specimens. It is less frequently met with than *L. mcgregori* Banks and therefore less likely to prove a menace.

*Paraiecanium cocophyllae* Banks.

*Paraiecanium cocophyllae* Banks, *Phil. Journ. Sci.* (1906), 1, No. 3, p. 235. (Pls. VIII to XI.)

This insect differs from any of the foregoing in that, if a female, it does not lie beneath a puparium but is itself its own scale. It has easily been found upon nearly every coconut examined in Manila, and is readily distinguished from other species by its unusual size (being 5 to 6 millimeters long and nearly as broad), and by the 2 small patches of orange-yellow on the posterior region. It always occurs upon the inferior surface of the leaf. The male puparia are much scarcer than those of the female.

A peculiar characteristic of the male insect is that it comes from beneath its scale to shed the pupal exuviae, returning after it has completed its transformation. The length of time after the final molt and before it seeks the female, during which the adult male remains beneath the puparium is not known.

## PREVENTIVES AND REMEDIES.

In all the work upon scale insects affecting the coconut, it has uniformly been observed that those trees which are ill cared for or which have become deformed by the attacks of beetles are the ones most infested by scales. The malformed or pathologically imbricated leaves, in their interstices, offer ideal places for the breeding of scale insects. This fact would point to the necessity of the removal and destruction of such portions at once.

Because scale insects can only migrate as wingless larvæ, it would seem that their arrest would not be difficult, and yet, when we consider that every wind blows these larvæ from leaf to leaf and from tree to tree, we can easily see that this fact, as well as the extreme fecundity of the insect, renders no tree entirely safe from their attacks. However, those trees which are the healthiest and best cared for are the ones which will best withstand these pests.

Spraying with lime-sulphur or kerosene emulsion washes might serve, if properly applied, for the preservation of young coconut trees, but these remedies would entirely be out of the question for full-grown ones. The necessity is apparent for clean, systematic and regular cultural methods for the protection of this valuable tree from scale, as well as from all other insect pests.

## INSECTS AFFECTING COPRA.

In connection with work which has been carried on in this Bureau on coconuts, copra, and coconut oil, it has been noted that certain lots of commercial copra, when received from the bodegas, were badly infested by larvæ, pupæ, and adults of *Silvanus surinamensis* Linn., and *Necrobia rufipes* De Geer. Both of these insects are cosmopolitan and as they

feed upon stored products of a character similar to copra it is only necessary to mention them in this connection.

In the case of these, and of most other injurious insect forms, preventive measures are always most advisable and if care is taken in packing and shipping the copra, receptacles into which the insects can not find entrance being employed, their ravages will be prevented. It is difficult to exterminate them if once they secure a lodgment in a mass of copra. Carbon bisulphide, which might be used successfully against similar insects in grain, would probably prove detrimental to copra owing to its power of dissolving oil.

## BIBLIOGRAPHY.

- BLANFORD, W. F. H. Palm Weevil in British Honduras, Royal Gardens, Kew, *Bul. of Useful Information* (1893), LXXIV, 27 et seq., plates 1 and 2.  
Reviewed by Riley & Howard, *Insect Life* (1893), V, 357.
- BUREAU, E. Calandra palmarum, *Ann. Soc. Ent. de France* (1853) XXVII, 1.
- CHITTENDEN, F. H. The Principal Household Insects of the United States, U. S. *Dept. Agr., Div. Ent.* (1896), IV, n. s., 105 and 121.
- COCKERELL, T. D. A. Coccidæ or Scale Insects, *Agric. Record* (1892), Dec. Reviewed by Riley & Howard, *Insect Life* (1893), V, 362.  
List of Coccidæ observed in Jamaica, *Insect Life* (1892), IV, 333.  
The West Indian Rufous Scale, *Ibid.* (1892), IV, 381.  
The Food Plants of Scale Insects, *Proc. U. S. N. Museum* (1896), 19, 780, *Cocos nucifera* L. Fifteen species of scale insects noted as occurring upon the coconut.  
Some Coccidæ from the Philippine Islands, *Proc. Davenport Acad. Sci.* (1905), X, 133.
- COCONUT, ENEMIES OF. *Trop. Agr.* (1889-90) IX (1890-1), X et vol. seq. Notes upon various injurious insects attacking coconut.
- COQUEREL, CH. Observations entomologiques sur divers Insects Recueillies à Madagascar. Sur les mœurs des Oryctes de Madagascar et sur deux espèces de Scolia qui vivent dépens des larves de ces Oryctes. *Ann. Soc. Ent. de France* (1855) III, 167.  
Faune de Bourbon, Ile de la Réunion, Coléoptères, *Ann. Soc. Ent. de France* (1866) VI, 334.
- CURTIS, J. (Ruricola) Aleurodes cocois, *Gardener's Chronicle* (May 2, 1846).
- DE MORNAY, C. F. Letter on Palm-beetles to *Penang Gazette and Straits Chronicle* (Sept. 24, 1889).
- FAWCETT, W. Report on the Cocoa-nut Disease at Montego Bay. *Bul. Bot. Dept. Jamaica* (Sept., 1891).  
Correspondence on the Cocoa-nut disease. *Ibid.* (May, 1892).
- FERGUSON, A. M. & J. All about the Coconut Palm (1885).
- FERNALD, MRS. M. E. A Catalogue of the Coccidæ of the World (1903).
- GREEN, E. ERNEST. Coccidæ of Ceylon (1896), 95, 207, 218.
- HICKEY, JOHN B. Notes on the Palm Weevil (partly reproduced from *Home and Farm*, Louisville, Ky.); also note by editors *Insect Life* (1891), 4, 136.
- HORN, G. H. Note on Larva of Rhynchophorus cruentatus. *Trans. Am. Ent. Soc.* (1878), VII, 39.
- KONIGSBERGER, J. C. Ziekten van Rijst, Tabak, Thee en andere cultuur gewassen die door Insecten worden veroorzaakt. *Meded. uit. s'Landsplanten. van Java* (1903), LXIV, 92 (1898), XXII, 31; 33, 39, 42.

- PHILLIPS, J. H.; GARR, F. E., & BELLAMY, J. *Report of Commissioners appointed by his Excellency the Governor to inquire into the destruction of the Cocoa-palm by the Palm-Weevil, with abstract of evidence given by Planters, Belize, British Honduras. (Jan. 18, 1889.)*
- PRESTON, HENRY. *Report on Cocoa-nut Diseases, Demerara Times (1876, March 16).*
- RIDLEY, H. N. *Report on Destruction of Coconut Palms by Beetles, Singapore (1889).*
- RILEY, C. V., & HOWARD, L. O. *An Enemy to the Date Palm in Florida, Insect Life (1888), I, 14.*  
*Bark Lice on the Cocoa-nut, Ibid (1889) I, 355.*  
*Notes on the Palm Weevil, Ibid (1891), IV, 136.*  
*The Coconut and Guava Mealy-wing, Ibid (1893), V, 5, 314.*
- RUSSELL, W. *The Cocoanut Palm: Its Culture and Diseases (1876).*
- SHORT, JOHN. *A Monograph of the Cocoanut Palm or Cocos nucifera, illustrated (1888).*
- SIGNORET, V. *Essai sur les Cochenilles, Ann. Soc. Ent. de France (1869) (4), IX, 120 and 121, Pl. XII, figs. 8 and 8 A.*
- SIMON, M. F. *Report on Beetles Injurious to Coconut Trees, Straits Settlements Government Gazette (1887, May 27). Tropical Agriculturist (1887), VII, 548.*
- STANTON, W. A., S. J., *Notes on Insects affecting the Crops in the Philippines. Some Insect Enemies of the Cocoanut Palm. Bul. Phil. Weather Bureau (August, 1903), 223.*
- SUMMERS, S. V. *Notes on Rhynchophorus zimmermanni Sch. Can. Ent. (1873), V, 123.*
- URICH, F. W. *Notes on Some Insect Pests of Trinidad, B. W. Ind. Insect Life (1894), VI, 196 et seq.*
- VERMONT, J. M. B., and KENNEDY, J. V. *Report by the Select Committee of the Legislative Council on the Coconut Trees Preservation Bill. Penang, Straits Settlements (Dec. 23, 1889).*
- "W. B. L." *Coconut Cultivation. Tropical Agriculturist (1884-5), IV, 1-3, 38-40, 375-376.*

#### ERRATA.

In Part I of this paper:

Pages 161, line 1, and 165, line 14, for Bonñga read Bonga.

Page 166, Table of Illustrations, for Plate I, fig. 1, adults of *R. ferrugineus* Fabr., read Plate I, fig. 1, egg of *Oryctes rhinoceros* L., magnified portion shown at 1b.

Plate VIII, fig. 3 should be reversed.



## ILLUSTRATIONS.

[All figures are more or less magnified; exact size is indicated in descriptions.]

### PLATE I.

Coconut leaf showing work of *Padraona chrysozona* Plötz. Note that on some of the leaflets the apical portion of the blade has been eaten, while in others the basal portion is represented by the midrib alone.

### PLATE II. Drawn by W. Schultze.

- FIG. 1. Coconut leaflet showing abandoned notches made by young larvæ of *Padraona chrysozona* Plötz.  
 2. Egg on margin of leaf.  
 2 A. Profile of egg.  
 3. Full-grown larva.  
 3 A. Profile of head.  
 4. Pupa.  
 4 A. Lateral view of pupa.  
 4 B. First thoracic spiracle or stigma.

### PLATE III. Drawn by W. Schultze.

- FIG. 1. *Padraona chrysozona* Plötz, male.  
 2. Female.

### PLATE IV.

- FIG. 1. *Chalcis obscurata* Walk., adult.  
 2. Coconut leaflet with cocoons of Braconid parasitic on *P. chrysozona* Plötz together with shriveled caterpillar skin of latter.

### PLATE V.

- FIG. 1. *Thosea cinereamarginata* Banks, full-grown larva.  
 2. Pupa.  
 2 A. Lateral view of pupa.

### PLATE VI.

- FIGS. 1, 1 A. Coconut leaflet with adult females and young of *Aspidiotus destructor* Sign. Note arrangement of young along veins.  
 2. Coconut leaflet attacked by disease causing spots similar to those produced by *A. destructor* Sign.

### PLATE VII.

- FIG. 1. *Aspidiotus destructor* Sign., adult male.  
 2. Female puparium, showing adult and eggs.  
 3. Male puparium.  
 4. Egg.  
 5. Antenna of larva, distal segment.  
 6. Pygidium of female.  
 7. Antenna of adult male, distal segment.  
 8. Young larva.

## PLATE VIII. Drawn by T. Espinosa.

- FIG.      1. *Scymnus* sp., adult.  
          2. Larva.  
          3. Antenna of adult.  
          4. Maxillary and labial palpi and labium.

## PLATE IX.

Coconut leaflet showing male and female puparia of *Chionaspis candida* Banks.  
Note group of male scales around female at lower right. A few male and female scales of *Chrysomphalus proprosimus* Banks occur also.

## PLATE X.

*Chrysomphalus proprosimus* Banks. Male and female puparia on leaflet of coconut.

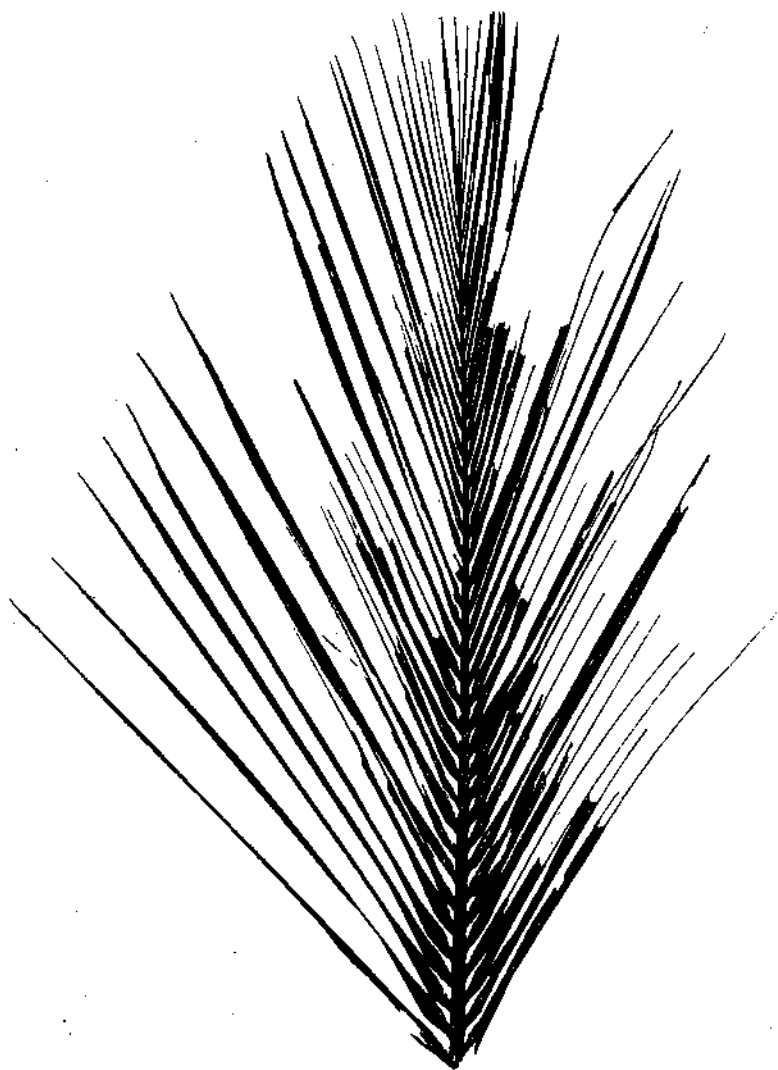


PLATE I.

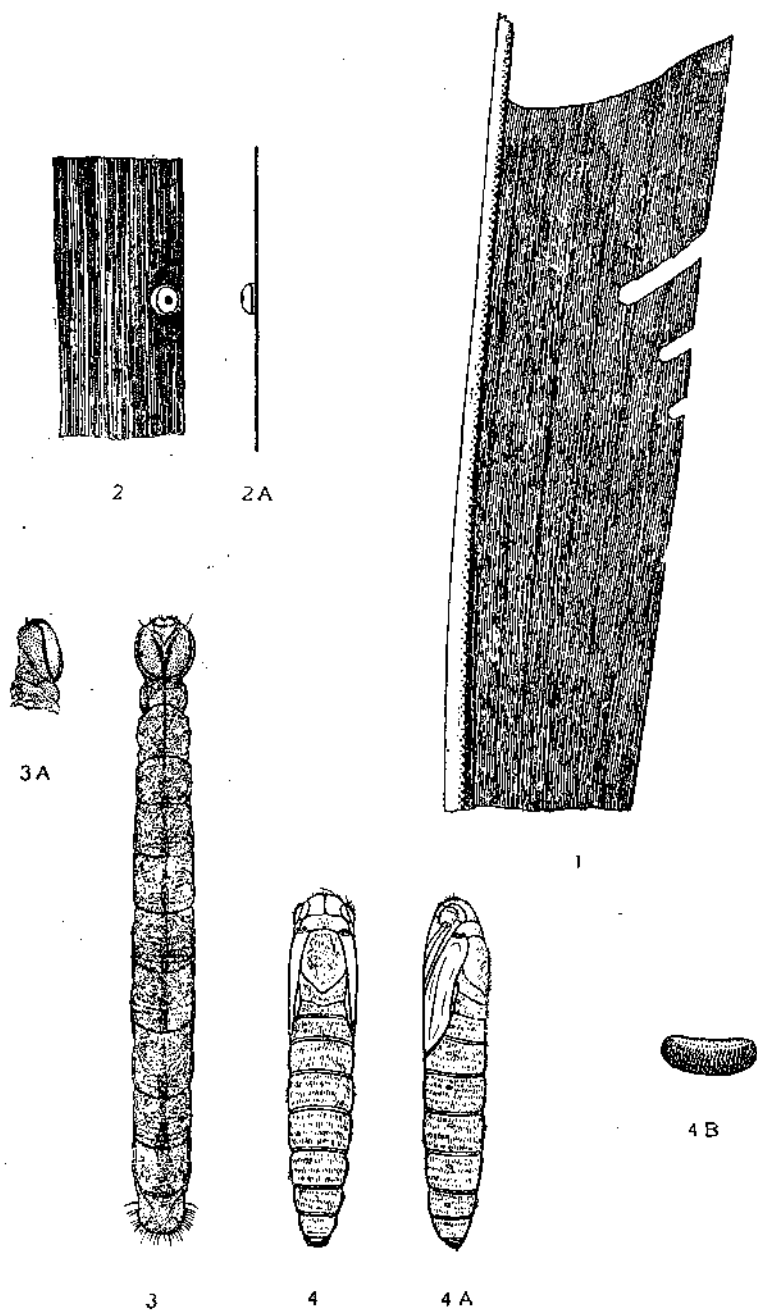
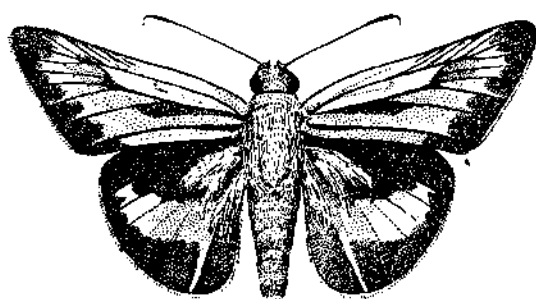
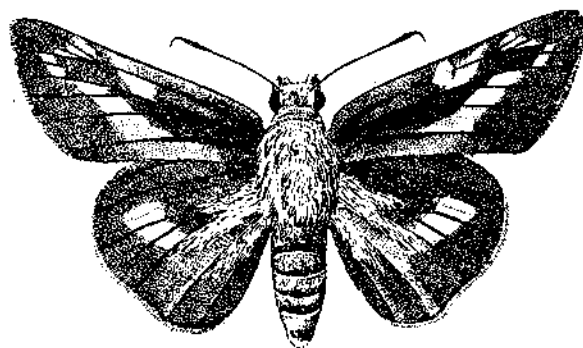


PLATE II.



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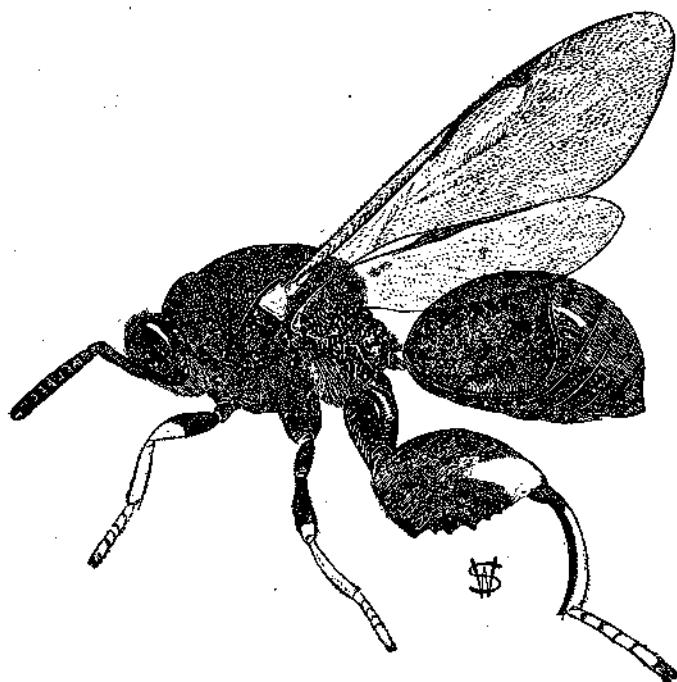
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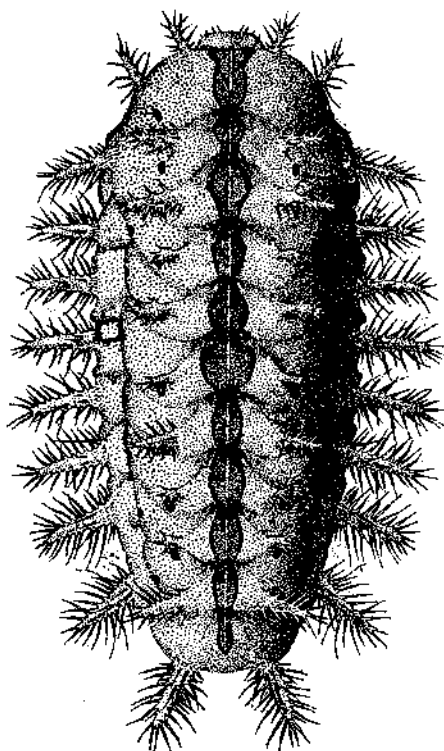
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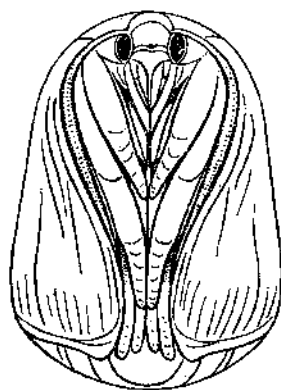
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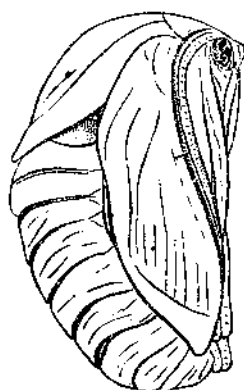
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PLATE V.



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PLATE VI.

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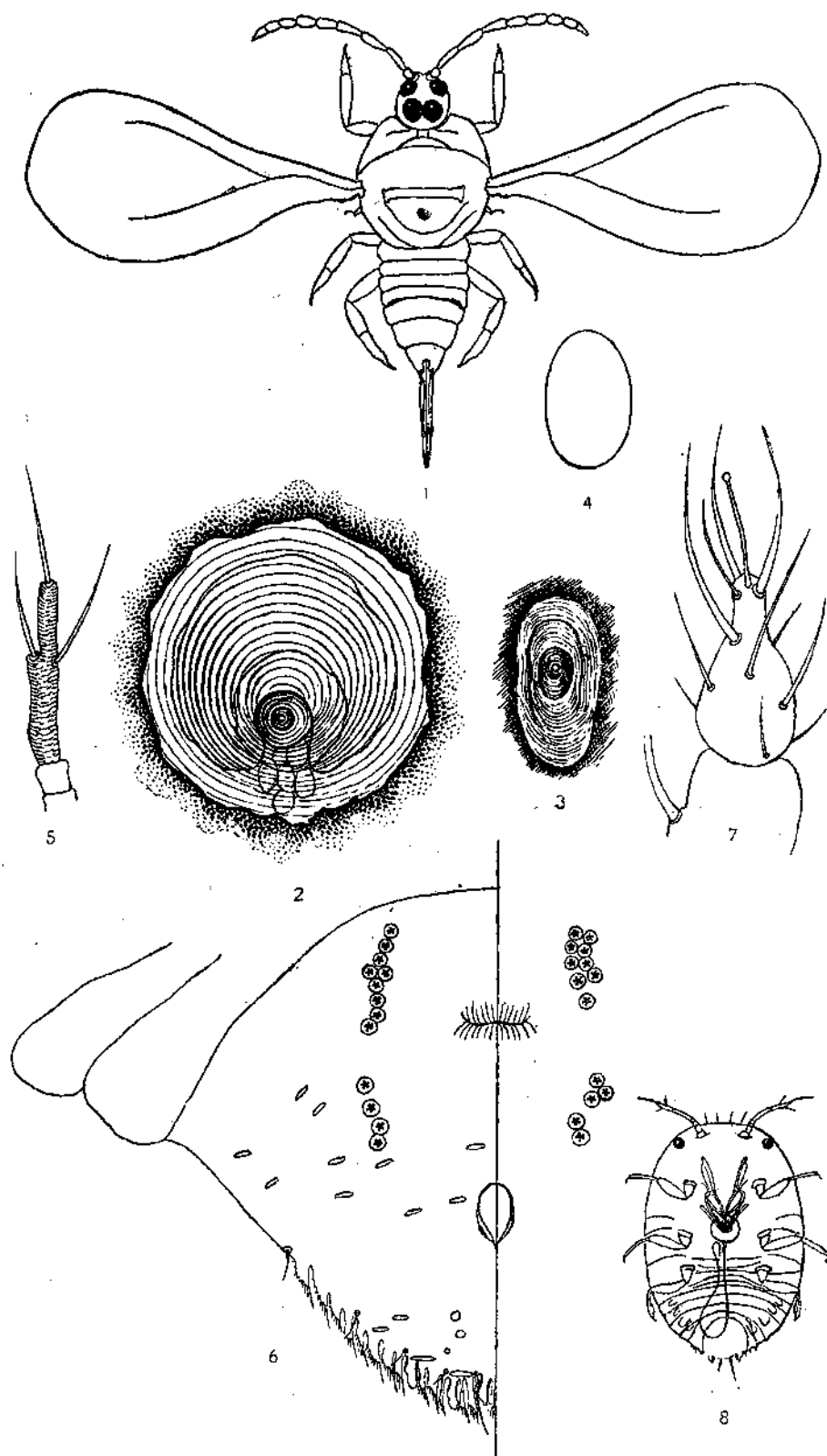
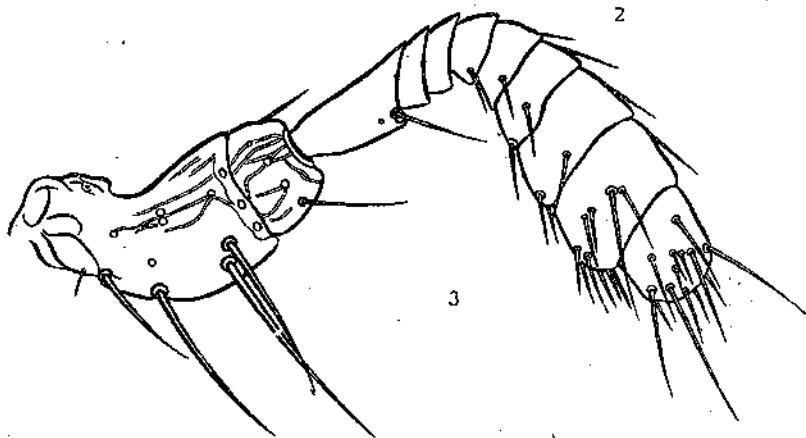
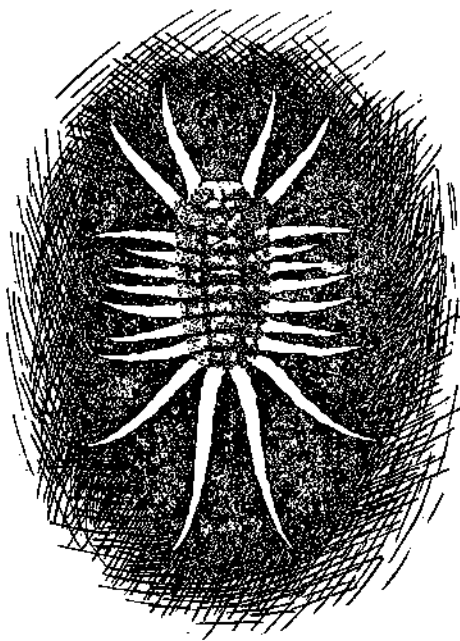


PLATE VII.



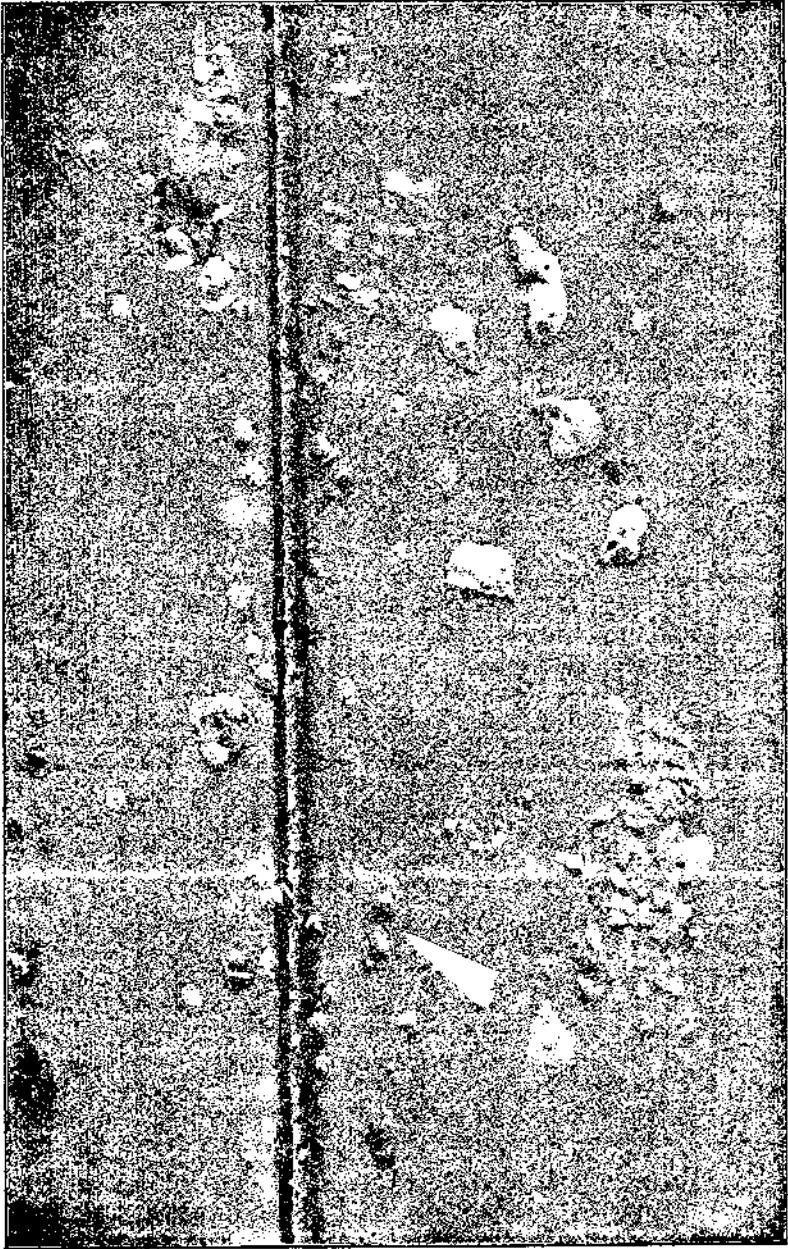


PLATE IX.



PLATE X.

## NEW PHILIPPINE INSECTS.

By CHARLES S. BANKS.

(From the Entomological Section of the Biological Laboratory, Bureau of Science.)

The insects described as new in this paper were encountered while I was pursuing investigations upon the ones which injure the coconut palm. With one exception, that of *Thosea cinereamarginata* n. sp., they belong to the Coccidæ. Although twelve species of the Limacodidæ, including three of *Thosea*, are recorded from the Philippines, this number is small in comparison with those from neighboring regions, Hampson recording 82 species in his Fauna of British India.

### LEPIDOPTERA.

#### LIMACODIDÆ.

##### *Thosea cinereamarginata* n. sp.

*Thosea*, Walk. Cat. (1855), 5, 1068.

Aphendale, Walk. Cat. (1865), 33, 494.

Anzabe, Walk. Cat. (1855), 5, 1093.

Male: Head, thorax, and abdomen dark-brown; latter transversely banded dorsally with lighter brown. Two dorsal and anal tufts dark-brown. Tegulae white-tipped. Fore-wing satiny dark-brown with purplish effulgence in certain lights. A moderately large darker spot at end of cell with a dark-brown oblique sinuate band beginning slightly posterior to it and extending to middle of inner margin; a similar, inward-curving, post-medial band beginning widely on costa before apex and extending nearly to posterior angle. Cilia attenuately spatulate, bronzed at tips and lighter than wing. Hind-wing grey-brown; veins and a discal spot slightly darker. Cilia slightly lighter. Under sides of wings grey-brown; fore-wing pearly at base and inner marginal area; hind-wing irrorated with dark-brown, scattered scales. Antennae darker at base; first joint with long cilia. Palpi pale anteriorly. Tibiæ, tarsi, and venter with long, grey hairs. Length, 12 millimeters. Length of wing, 9.5 millimeters.

Female: Head, thorax, and abdomen brown-grey, latter transversely marked on apical dorsal margins of 5 to 7 segments with light ochraceous hairs. Fore-wing, except outer fourth, purplish brown-grey. Dark-brown spot at end of cell and an oblique dark-brown band as in male but much more distinct. Post-medial band parallel with outer margin for one-half its length, then curved inward and ending before posterior angle. Marginal area mouse-grey, irrorated with few, dark-brown scales. Cilia and hind wings as in male but latter with a light, clearly defined marginal line. Anal tuft with black-tipped hairs. Under side of wings

as in male. Legs with white-tipped, grey hairs. Antennae and palpi dark-bronze brown. Length 12.5 millimeters, length of wing 11.25 millimeters.

Habitat: Manila (Banks Coll.).

On *Cocos nucifera* L.

Types of male and female: No. 4888 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

#### HEMIPTERA.

##### Coccidæ.

##### DIASPINÆ.

#### *Chrysomphalus prosimus* n. sp.

*Chrysomphalus* Ashm., *Am. Ent.* (1880), 3, 268.

Orange Ins. (1880), 21.

Leon., *Riv. Pat. Veg.* (1899), 7, 198.

Aonidilla Berl. e Leon., *Riv. Pat. Veg.* (1895), 4, 77.

Idem. (1899), 7, 174. Type aurantii.

s. g. *Melanaspis* Ckll., *Bul. T. s. U. S. Dep. Agr.* (1897), 6, 5. Type, obscurus.

s. g. *Mycetaspis* Ckll., *Bul. T. s. U. S. Dep. Agr.* (1897), 6, 5. Type, personatus.

*Inaspidiotus* Barreda, *Boll. Com. Parasit. Agr.* (1901), 229.

*Chrysomphalus* Fernald, *Cat. Coc. World* (1903), 285.

Type: ficus.

Female puparium (Pl. I, fig. 2) circular, convex, 1.5-2.5 millimeters in diameter, dark-chocolate brown, grayish at margin. Pellicles (Pl. II, fig. 2), dark-golden orange, covered with white, waxy film in perfect specimens; central or subcentral; a small tubercular prominence at center of first pellicle, surrounded by slight depression. Margins of pellicles lighter than disc, which is scabrous. Ventral scale exceedingly thin, white, waxy; often remaining intact upon leaf after upper scale has dropped off and giving the surface a moldy appearance. Male puparium (Pl. I, fig. 3), 0.75-0.90 millimeter long, obtuse-oval in outline; of same general color as female; pellicle slightly eccentric or near one extremity and dark-orange. Adult female (Pl. II, fig. 1): 1.05 millimeters long, 0.90 millimeter wide, nearly circular in outline, except posterior area, which is sharply prolonged to apex of pygidium; yellow; mesothoracic margin very slightly thickened and bearing a spine, minutely denticulate at its base. (Pl. II, fig. 4.) Pygidium (Pl. II, fig. 3) acute laterally; the 6 lobes subequal in size and each having an acute exterior tooth; squames deeply compound and fimbriate, 2 in mesal, first and second spaces, and 3 in third space beyond third lobe. Beyond last squames, margin is thickened and minutely denticulate, dentations in a continuous group, thus differing from *Chrysomphalus aonidium* Linn., to which the scale is most closely related. Margins of abdominal segments quite markedly lobed, each bearing a spine. Two spines at base of pygidium on each side. Circumgenital glands in 4 groups, the posterior with 4 and the anterior with 6 to 8 orifices. Genital aperture between posterior pair of glands. Dorsal pores of tubular segments in 2 subparallel series on each side, containing 18-23 orifices, extending halfway to base of pygidium. Entire margin of insect exterior to pygidium, minutely serrate, with slender spines at irregular intervals. Anterior spiracles opposite middle of rostrum, posterior halfway between rostrum and base of pygidium.

Adult male (Pl. I, fig. 1) 0.88 millimeter long, including genital style, very stout; wing 0.60 millimeter long, yellow; apodema darker; eyes crimson-red and occupying nearly entire head. Antennae nearly as long as body, exclusive of the

style which is 0.27 millimeter long; antennal segments having long hairs; tarsal claw long, slender, spiniform with apex curved.

Eggs, yellow, 0.15 millimeter long.

Young: Yellow, 0.18-0.20 millimeter long, 0.13 millimeter wide.

Habitat: Philippines, Manila; San Miguel de Mayumo, Prov. of Bulacan (Banks Coll.).

On leaves of *Cocos nucifera* L., both surfaces.

Type: No. 10164 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species is so like *C. aonidium* L., that I hesitated to call it new, but the color of the puparia and pellicles of male and female and the difference in pygidial characters make the necessity for its separation as a new species quite evident.

*Pariatoria greeni* n. sp.

*Pariatoria* Targ., Catalogue (1869) 42.

——— Sign., *Ann. Soc. Ent. Fr.* (1869) (4) 9, 450.

——— Green, *Coccidæ Ceylon* (1899), 162.

——— Fernald, *Cat. Coc. World* (1903) 318. *Et al. in litt.*

Type: *lucasii* = *ziziphi*.

Female puparium (Pl. III, fig. 1) pale to dark-slate color, lighter at margins; of a regular, broad-oval; 1.35 to 1.65 millimeters long, 0.90 to 1 millimeter wide. First pellicle broad-oval, narrow in front; of a transparent, yellow-ocher, having a broad, emerald-green, median, carinated band from front to hind margins. Second pellicle circular, except for squames at posterior extremity; of same color as first pellicle; with a broad, subtriangular emerald-green median band from anterior margin to disc and transverse, oval patch of the same color at posterior extremity. Pellicles at anterior extremity of puparium and occupying, in old specimens, slightly less than one-half total length. Their axes seldom in line with main longitudinal axis of puparium. Ventral scale a mere flange one-sixth the width of the puparium.

Male puparium (Pl. III, fig. 2) 0.87 millimeter long, 0.26 millimeter wide, of a dirty-white; sides parallel, carinate; posterior margin rounded, posterior area flattened. Pellicle similar to first pellicle of female, tipped forward at an angle of 45 degrees.

Adult female (Pl. III, figs. 4 and 4 A) (living specimen): Broadly elliptical in outline; head tapering; body broadest across posterior three-fourths. Length 0.60 millimeter, width 0.45 millimeter, somewhat smaller than second pellicle, beneath which it lies. Discal area swollen. Marginal area flattened. Margin minutely serrated. Abdominal segments quite distinct, less so at margin. Disc, dark-purple, marginal area lighter, almost white in certain points. Pygidium pale-ocher tinged with purple. Anterior pair of spiracles slightly posterior to line across base of rostrum and each two-thirds of distance from rostrum to margin; posterior pair, each at a point posterior to apex of rostrum, equal to length of latter and one-third distance from median line to margin. Skin transversely papillate. Tip of rostrum ocher, base paler, nearly white, length of rostral setæ one and one-half times that of body. Margin bears on each side isolated spines not exceeding 6, and a spinous tubercle at a point opposite apex of rostrum.

Pygidium (Pl. III, fig. 6) evenly rounded posteriorly. Lobes and squames (Pl. III, fig. 5) of about equal length, tips of squames very slightly longer. Six principal lobes subequal in size, tridentate. Rudimentary lobes distinct, cuspid, one-half the length of principal lobes. Order of arrangement of lobes, squames, and spines from mesal space as follows: 1 squame, 1 lobe, 2 squames, 1 lobe, 1 squame,

1 spine, 2 squames, 1 lobe, 1 spine, 1 squame, 1 spine, 2 squames, 1 rudimentary lobe, 1 squame, 1 spine, 2 squames, 1 rudimentary squame, 1 rudimentary lobe, 1 squame, 1 spine, 1 squame, beyond which a series of modified cuspid lobes on margins of second and third abdominal segments. Marginal semi-lunar pores prominent, 9 on each side, one median; first 2 on each side subtend 2 squames, remainder not as wide as 2, but wider than 1 squame. A submarginal series of dorsal oval pores more numerous and thickly placed near base of submarginal area of pygidium. Circumgenital glands in 4 groups of which the posterior pair contain each 5 openings and the anterior 6. This is quite constant. Anal orifice submarginal on dorsum.

Adult male not known.

Eggs (Pl. III, fig. 3) dark purple 0.28 millimeter long and 0.15 millimeter wide, placed transversely beneath scale.

Habitat: Philippines, Manila (Banks Coll.).

On upper surface of leaves of *Cocos nucifera* L.

Type: No. 10105 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species is most closely related to *Parlatoria proteus* Curt., but also resembles *P. pergandii* Comst., from which it differs in the color of the scale, *P. pergandii* Comst. being of a light yellow, transparent hue, while *P. greeni* n. sp. is slate color. The fourth lateral (first rudimentary) lobes are more developed and have dentate margins and a sharp terminal cusp. The color of the second pellicle is constant and characteristic.

This species is named in honor of my friend, Prof. E. Ernest Green, Government Entomologist of Ceylon, whose indefatigable labors in the study of the Coccidae are known and appreciated in all parts of the world.

*Chionaspis candida* n. sp.

*Chionaspis* Sign., *Ann. Soc. Ent. Fr.* (1869), (4) 9, 442.

Fernald, *Cat. Coc. World* (1903), 203. *Et al. in litt.*

Type: *salicis*.

Female puparium (Pl. IV, fig. 1) snow-white, 2.35-2.50 millimeters long, 1.15-1.23 millimeters wide. Considerably dilated posteriorly; narrow anteriorly, the sectionary area usually reaching middle of second pellicle, but sometimes to its anterior margin. Surface smooth, somewhat glabrous and showing only transverse lines of growth. Pellicles pale, fulvous, second slightly darker posteriorly. First pellicle, one-half the length and one-third the width of second, which it overlaps for one-half its own length. An almost imperceptible carina on second pellicle. Length of second pellicle 0.77 millimeter.

Male puparium (Pl. IV, fig. 2) snow-white, 0.85-0.90 millimeter long, 0.38 millimeter wide, with ill-defined median and lateral carinae. Surface woolly. Pellicle one-third length of puparium and of a very pale-fulvous.

Adult female (Pl. IV, fig. 3) length 1.25 to 1.75 millimeters, width 0.65 to 0.80 millimeter; pale-yellow, pygidium with brownish tinge posteriorly. Form, elongate oval. Antennae with basal knob, cuspid internally; apical joint stout, fleshy, quite similar in shape and size to larger pygidial squames, submarginal, on anterior part of head. Entire surface of body minutely palpillate. Dorsum, posterior to a transverse line through apex of rostrum, marked by 2 submedian subparallel, linear stomata diverging posteriorly. Abdominal segments acutely lobed (Pl. IV, Fig. 3). Anterior spiracles having well-defined parastigmatic glands and removed laterally from apex of rostrum a distance equal to width of latter at base. Posterior spiracles slightly smaller than anterior and placed a



little before middle of body, about equidistant from each other and from the margin. Pygidium (Pl. IV, fig. 4) with circumgenital area marked by a horse-shoe of snow-white secretion, open posteriorly. Median lobes projecting one-third their length from an obtuse posterior notch; their interior margins minutely dentate (usually 5 dentations). External cusp rounded; laterad of this a small tooth; a pair of spines between median lobes. Exterior to median lobe a spinose squame, a marginal pore, 2 lateral lobes, the larger dentate laterally, and their margins rounded. Exterior to lateral lobes, a squame, 2 marginal pores; on dentations of margin, a squame, 2 marginal pores, a squame, 2 marginal pores, a squame, a marginal pore and 6 squames, latter on third abdominal segment. Segmental areas quite well defined by plications and series of dorsal pores, the latter forming on each side 4 distinct series as follows: On second abdominal segment 8 submarginal; on third, 4 submedian and 8 submarginal; on fourth, 3 submedian and 5 submarginal; on pygidium, 2 submedian, 5 submarginal and 1 exterior to anterior group of circumgenital glands. Cylindrical ducts short and stout. Circumgenital glands arranged in 5 groups, the median with 8, the anterior lateral with 14-19 and the posterior with 14-16 openings. Anal opening directly above genital—i. e., both lie in same vertical plane.

Adult male not known.

Eggs pale-yellow 0.30 millimeter long and 0.15 millimeter wide. Plainly visible through body wall of female.

Larvæ (Pl. IV, fig. 5) pale-yellow, 0.40 millimeter long upon emerging from egg.

Habitat: Philippines, Manila (Banks Coll.).

On upper surface of leaves of *Cocos nucifera* L.

Type No. 10102 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

**Lepidosaphes mcgregori** n. sp.

*Lepidosaphes* Shimer, *Tr. Am. Ent. Soc.* (Jan, 1888), 1, 373.

*Mytilaspis* Sign., *Ann. Soc. Ent. Fr.* (1888), (4) 9, 91; not described.

*Phaulomytilus* Leon., *Riv. Pat. Veg.* (1897), 6, 205, 206.

*Coccoomytilus* Leon., *Riv. Pat. Veg.* (1897), 6, 205, 206.

*Trichomytilus* Leon., *Riv. Pat. Veg.* (1897), 6, 205, 206.

*Lepidosaphes* Fernald, *Cat. Coc. World* (1903), 304.

Type: *conchiformis* = *ulmi*.

Female puparium (Pl. V, fig. 1) 2.50-2.65 millimeters long, 0.60-0.75 millimeter wide; very long and narrow, with the sides gradually diverging posteriorly and well rounded dorsally; clear, red-brown, with lighter to whitish margins; transverse striæ separated into groups by more pronounced carinæ. First pellicle broadly oval, yellow-ochraceous, with broad, median carina and 2 waxy, anterior horn-like projections; second pellicle slightly darker, more elongate, narrowing abruptly and acutely posteriorly, hind margin and an anal patch light ochraceous, with a transverse discal carina and a decided spine on margin of second abdominal segment.

Male puparium (Pl. V, fig. 2) 1.45 millimeters long, 0.35 millimeter wide, narrow anteriorly and widening behind; posterior margin subcircular. Pale, yellow-brown anterior, waxy, horn-like projections lacking; posterior and lateral margins narrowly white; a transverse, pre-anal, thin portion of scale marking portion which may be elevated for escape of adult male.

Adult female (Pl. V, fig. 5) 0.90-0.95 millimeter long, 0.35 millimeter wide, of a light-yellow, almost white; the anterior extremity slightly narrower, widest part just anterior to pygidium. Antennæ halfway between front margin and base of rostrum and having each an axillary spine mediad, nearly equal to them

in length. Anterior spiracles opposite apex of rostrum and halfway to lateral margin, with a few stigmatic orifices anteriorly. Mentum subtriangular. Posterior spiracles conspicuous. Abdominal segments easily differentiated and strongly lobed at margins; first segment spineless, second with 3, third and fourth with 4 spines each. Thoracic cuticle not especially chitinous; irregularly papillate. Pygidium (Pl. VI, fig. 3) narrower than long, pale-ochraceous, slightly darker than abdominal margins. Anal orifice three times its own diameter, from anterior margin of pygidium, protected on each side by a longitudinal fold, the 2 converging posterior to anus and exteriorly to disc of pygidium in parallel lines. Circumgenital glands in 5 groups, the median with 4 orifices, the anterior lateral with 6 and the posterior with 5 to 6. The grouping is not well defined. Median lobes broad, their surfaces crenulate, rounded, and having a distinct tooth at each side; second pair of lobes double, their margins straight. A median, acute tubercle in mesal space, with a long spinous squame on either side; 2 squames between first and second double lobes; 2 beyond second double lobes, 2 groups of 2 each toward base of pygidium. Margin of pygidium beyond lobes thickened and minutely serrated, with 3 prominent teeth, in the first 2 of which are 2 oval, glandular orifices, in the basal of which, one. Another similar orifice between median and second lobes, making total of 6 for each side. Genital orifice one-third distance from median group of circumgenital glands to posterior margin. Numerous, circular, dorsal pores irregularly arranged, 2 being submedian posteriorly, 1 below each of second lobes and remainder near center of disk.

Adult male not known.

Eggs (Pl. V, fig. 3) 0.28 millimeter long, 0.11 millimeter wide, of a rather light purple, very regularly arranged in puparium (Pl. V, fig. 4).

Larva (Pl. VI, fig. 2) 0.29 millimeter long, 0.11 millimeter wide, slender, somewhat polygonal, bright-yellow, having a prominent spine on posterior, lateral margin of mesothorax. Antennae slender, transversely striate, with few hairs, 2 stout ones at tip; a few spines on posterior and anterior extremities, 2 submedian caudal setae one-third length of body. Tarsal knobbed hairs twice length of unguis.

Habitat: Philippines, Manila (Banks, Coll.).

On both surfaces of leaves of *Cocos nucifera* L.

Type: No. 10142 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species differs from *L. cocculi* Green in having no cephalic transverse fold, in the ventral scale being incomplete, in the color and disposition of the eggs, and in the form of the median lobes. In addition, the general appearance of the scale and the perceptible prolongation of the anterior, waxy, horn-like projections readily differentiate it as a separate species.

I take pleasure in naming this species for my friend Richard C. McGregor, a most diligent collector and an accurate observer of insect life in the Philippines.

#### *Lepidosaphes unicolor* n. sp.

Female puparium (Pl. VII, fig. 1) 1.80-1.90 millimeters long, 0.50 millimeter wide, of about the same general shape and size as *L. mcgregori* n. sp., except that fore end of first pellicle is more acute and marginal flattening is hardly perceptible; of a dark-red or dark-cherry-brown including both pellicles, except a lighter patch on sides and caudal end of second. Both pellicles with median carina, puparium transversely multicarinate (usually 7 carinae), the carinae sep-

arating transversely-striate, paler areas. At posterior end there is a pair of subparallel, longitudinal elevations or carinae. This feature appears to be constant.

Male puparium not known.

Adult female (Pl. VII, fig. 3) 0.90 to 0.05 millimeter long, 0.35 millimeter wide, of a pale, transparent, cream-color, nearly white, narrow anteriorly, widening uniformly to third abdominal segment, then somewhat abruptly rounding to extremity. Front rounded; latero-frontal angles of head produced laterally into spinose, concentrically papillate knobs. (Pl. VII, fig. 4.) Antennae nearer front than base of rostrum; secondary spine as long as primary and usually strongly curved. First spiracles opposite mentum, nearer margin. Abdominal segments strongly produced laterally, then posteriorly. The second, third, and fourth with 3 to 4 stout spines and the second with a lateral cusp. Pygidium pale-ochraceous. Circumgenital glands hardly separated into groups, but consisting of an anteriorly curving series of 24 orifices, the median 4 of which are slightly separate from the laterals. Anal orifice a little more than its own diameter from base of pygidium; genital one-third the distance from the median group of glands to caudal margin. Median lobes as in *L. mcgregori* n. sp. Second double lobes with margins more rounding. Squames pseudo-fimbriate, the single fimbriation indicated on each by mere acute tubercle. (Pl. VII, fig. 6.) A median acute tubercle is present between mesal squames. Marginal orifices, on each side, with cylindrical spinnerets long and narrow; neither as large as in *L. mcgregori* n. sp. On dorsal area of pygidium are irregularly placed oval discal pores which are quite prominent in all specimens.

Adult male not known.

Eggs (Pl. VII, fig. 5) snow-white, 0.215 millimeter long, 0.10 millimeter wide, somewhat conical in form and laid in regular rows beneath puparium.

Larvæ (Pl. VII, fig. 7) pale-yellow, 0.23 millimeter long, 0.11 millimeter wide. Rather stout oval; front with 4 curved setæ, mentum circular, antennae moderately stout, setose. Apices of tibiae larger than bases; caudal margin with 6 spines on each side.

Habitat: Philippines, Manila (Banks, Coll.).

On upper surfaces of leaves of *Cocos nucifera* L.

Type: No. 10171 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This scale is quite distinct in appearance from *L. mcgregori* n. sp. Its color is uniform and the transverse carinae are fewer and less prominent on the puparium. The 4 irregularly placed discal pores of pygidium are not present in *L. mcgregori* n. sp. The great length and shape of the lateral abdominal margins and the shape of the squames serve to differentiate this as a new species.

*Paralecanium cocophyllæ* n. sp.

s. g. *Paralecanium* Ckll. & Parr., *The Industrialist* (1899) 277.

———— Ckll., *Can. Ent.* (1901) 33, 57.

g. ————— Ckll., *Ann., Mag. Nat. Hist.* (1902) (7) 9, 455.

———— Fernald, *Cat. Coc. World* (1903) 199.

Type: frenchii.

Adult female (Pl. VIII, fig. 1) 4-5 millimeters long, 3.5-4 millimeters wide; of a broad oval, almost circular and very slightly narrower anteriorly; margin with shallow, obtuse notches at stigmatic and anal clefts. Of a pale, transparent yellow; a distinct marginal area which is applied to leaf and which has its interior border marked by a thin, golden line. An orange-yellow patch on each

side of posterior area marking position of ventral egg cavities. These cavities lined with an orange-colored, viscid substance which soon dries upon exposure. A submarginal translucent area and a similar median one through which movements of internal organs are visible. An irregular, broken line of dark-brown spots, internally, shows through dorsal wall and probably marks lower part of alimentary canal. Dorsal surface minutely punctuate, and covered with a very thin, waxy substance in addition to a well-defined, longitudinally curved series of triplicate, polygonal, waxy laminae, the smaller of each of which is uppermost. These laminae are arranged as follows: A median series of 6 (sometimes the posterior is divided to form a seventh), a submedian of 9, a submarginal of 13 to 16 and a marginal of 28 to 30, on each side. (Pl. VIII, fig. 1.) The laminae mark the insect's stages of growth. (Pl. X, fig. 4.) Regularly arranged suboval pores over entire dorsum (Pl. VIII, fig. 2), but on each side of anal plate there is an outwardly and anteriorly curving series of 4 groups of pores. (Pl. VIII, fig. 7.) Marginal area with reniform, radiating cells arranged like scales on a fish. Anal opercular scales (Pl. VIII, fig. 5) triangular, pointed; the free edge being about equal to or slightly longer than base. Stigmatic clefts circularly incised at margin and invariably with 3 long, stout, blunt, curved spines (Pl. X, fig. 2), these spines not attaining outer margin, being removed their own length therefrom. Margin with series of flabelliform scales slightly overlapping (Pl. VIII, fig. 4), which are wider in proportion to their length than those of *P. (Lecanium) expansum* Green. Between the bases of flabellae, the margin is minutely dentate, the number of dentations being nonuniform. Eyes, red-brown, shining, placed two-thirds distance from rostrum to margin. Antennae (Pl. VIII, fig. 3) indistinctly 3 jointed, placed nearly in a line with and between rostrum and eyes at 3 times their own length from latter. Legs wanting. Genital orifice (Pl. VIII, figs. 5 and 6) obovate, anterior to anal apericulum at a distance equal to length of latter; containing an interior series of small and an exterior of large glands or pores. Exterior to orifice on ventrum minute spinnerets in 4 ill-defined groups on each side.

Male puparium (Pl. IX, fig. 2) 2.27 millimeters long, 1.20 millimeters wide, elongate-oval, silicious or waxy in structure, more convex than female and consisting of 17 plates, the posterior median being double the length of the anterior, thus differing from that of *P. expansum* Green in which the posterior median plate is divided, forming 2, or a total of 18. Upon each plate a series of widely separated waxy laminae, indicating larval covering. (Pl. X, fig. 4.)

Adult male (Pl. IX, fig. 1), length, including genitalia, 1.55 millimeters; length of wing 1.18 millimeters, of a uniform, pale yellowish-ocher, apodema darker-ocher. Eyes bright crimson. Antennae somewhat thickly and evenly setose from base to apex and with 3 knobbed hairs on last segment. Nervures of wings slightly darker than membrane. Legs as setose as antennae; tarsal claw acutely conical.

Eggs (Pl. XI, fig. 2) pale-yellow, 0.38 millimeter long.

Larvæ (Pl. XI, fig. 1) of same color as eggs, 0.48 millimeter long, 0.26 millimeter wide, the caudal setæ one-seventh length of body. Eyes brown-red, marginal. Anal cleft strong and deep (Pl. X, fig. 1.)

Habitat: Philippines, Manila (Banks, Coll.).

On under sides of leaves of *Cocos nucifera* L. in fair abundance.

Type: No. 10141 in Entomological Collection, BUREAU OF SCIENCE, Manila, P. I.

This species differs from *P. expansum* Green chiefly in the color, the shape of the flabellae, and the number of spines in stigmatic cleft, in the female, in the number of plates in the male puparium and the color of the apodema of the male.

## ILLUSTRATIONS.

[All figures are highly magnified; exact size is indicated in descriptions.]

### PLATE I.

- FIG. 1. *Chrysomphalus propositus* Banks, adult male.  
2. Female puparium.  
3. Male puparium.

### PLATE II.

- FIG. 1. *Chrysomphalus propositus* Banks, adult female.  
2. First and second pellicles.  
3. Pygidium of female.  
4. Lateral spine of female.  
5. Egg.

### PLATE III.

- FIG. 1. *Parlatoria greeni* Banks, female puparium.  
2. Male puparium.  
2 A. Lateral view showing angle at which pellicle is inclined.  
3. Egg.  
4. Adult female.  
4 A. Lateral view.  
5. Lobe and squame of pygidium.  
6. Pygidium of female.

### PLATE IV.

- FIG. 1. *Chionaspis candida* Banks, female puparium.  
2. Male puparium.  
3. Adult female.  
4. Pygidium of female.  
5. Larva immediately after hatching.

### PLATE V.

- FIG. 1. *Lepidosaphes mcgregori* Banks, female puparium.  
2. Male puparium.  
3. Egg.  
4. Under surface of female puparium showing adult, eggs and eggshells;  
latter toward posterior extremity and closely compacted.  
5. Adult female.

### PLATE VI.

- FIG. 1. *Lepidosaphes mcgregori* Banks, second pellicle.  
2. Larva immediately after hatching.  
3. Pygidium of female.

## PLATE VII.

- FIG. 1. *Lepidosaphes unicolor* Banks, female puparium.  
 2. First and second pellicle.  
 3. Adult female.  
 4. Frontal margin of female, showing antennæ and antero-lateral prominences or knobs.  
 5. Egg.  
 6. Pygidium of female.  
 7. Larva, immediately after hatching.

## PLATE VIII.

- FIG. 1. *Paralecanium cocophyllæ* Banks, adult female.  
 2. Magnified portion of derm, showing irregularly oval cells.  
 3. Antenna of female.  
 4. Marginal flabellæ.  
 5. Genital orifice and anal operculum.  
 6. Genital orifice, highly magnified.  
 7. Operculum of female showing curving series of pellucid glands extending toward margin.

## PLATE IX.

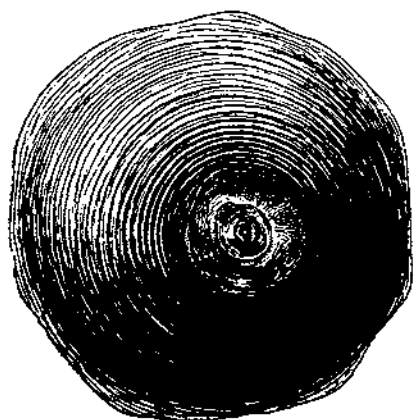
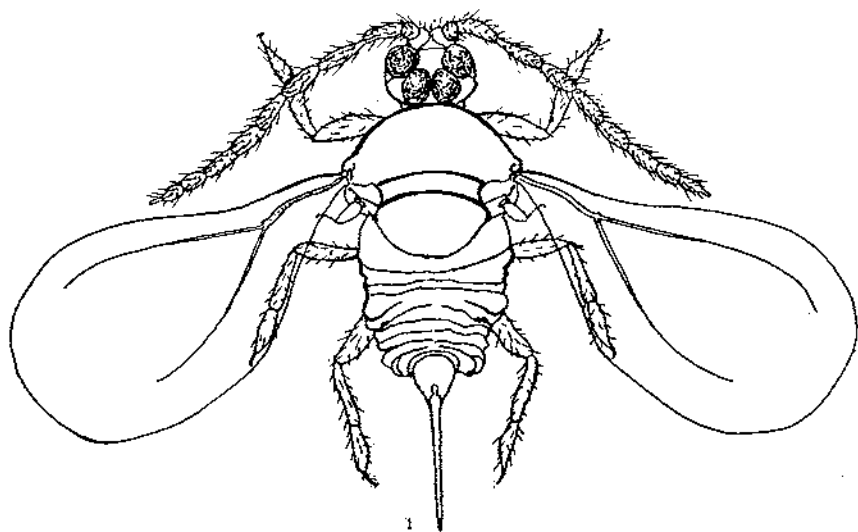
- FIG. 1. *Paralecanium cocophyllæ* Banks, adult male.  
 2. Male puparium showing superposed laminæ.  
 3. Male pupa.

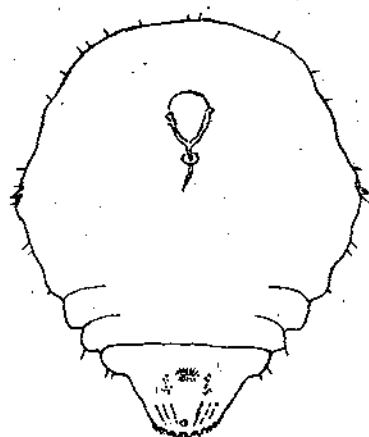
## PLATE X.

- FIG. 1. *Paralecanium cocophyllæ* Banks, caudal extremity of larva.  
 2. Stigmatic cleft of female with spines and marginal flabellæ.  
 3. Mouthparts of female.  
 4. A single division or plate of male puparium showing dorsal laminæ of larva, separated by growth of the insect. The sides which were formerly proximate are numbered from 1-20.

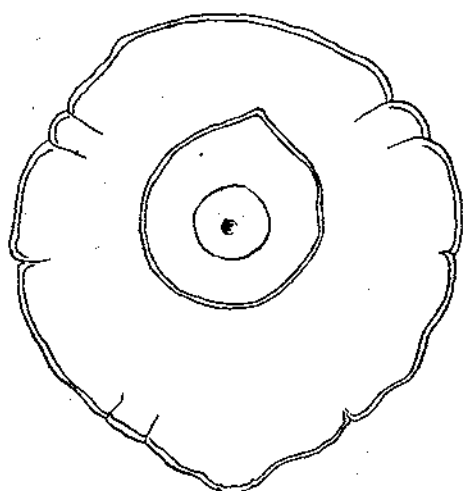
## PLATE XI.

- FIG. 1. *Paralecanium cocophyllæ* Banks, larva immediately after hatching.  
 2. Egg.  
 3. Antenna of larva.  
 4. Anterior leg of larva.  
 5. Middle leg.  
 6. Posterior leg.

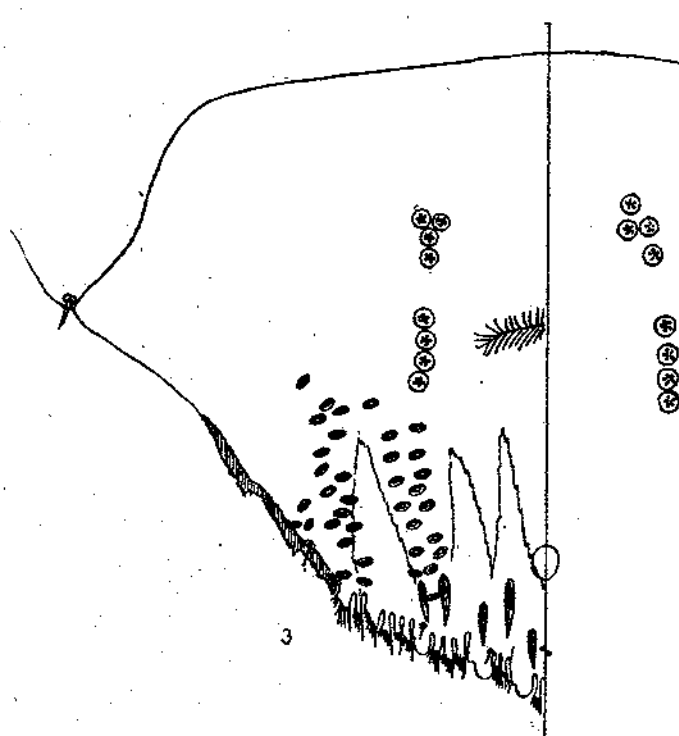




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PLATE II.



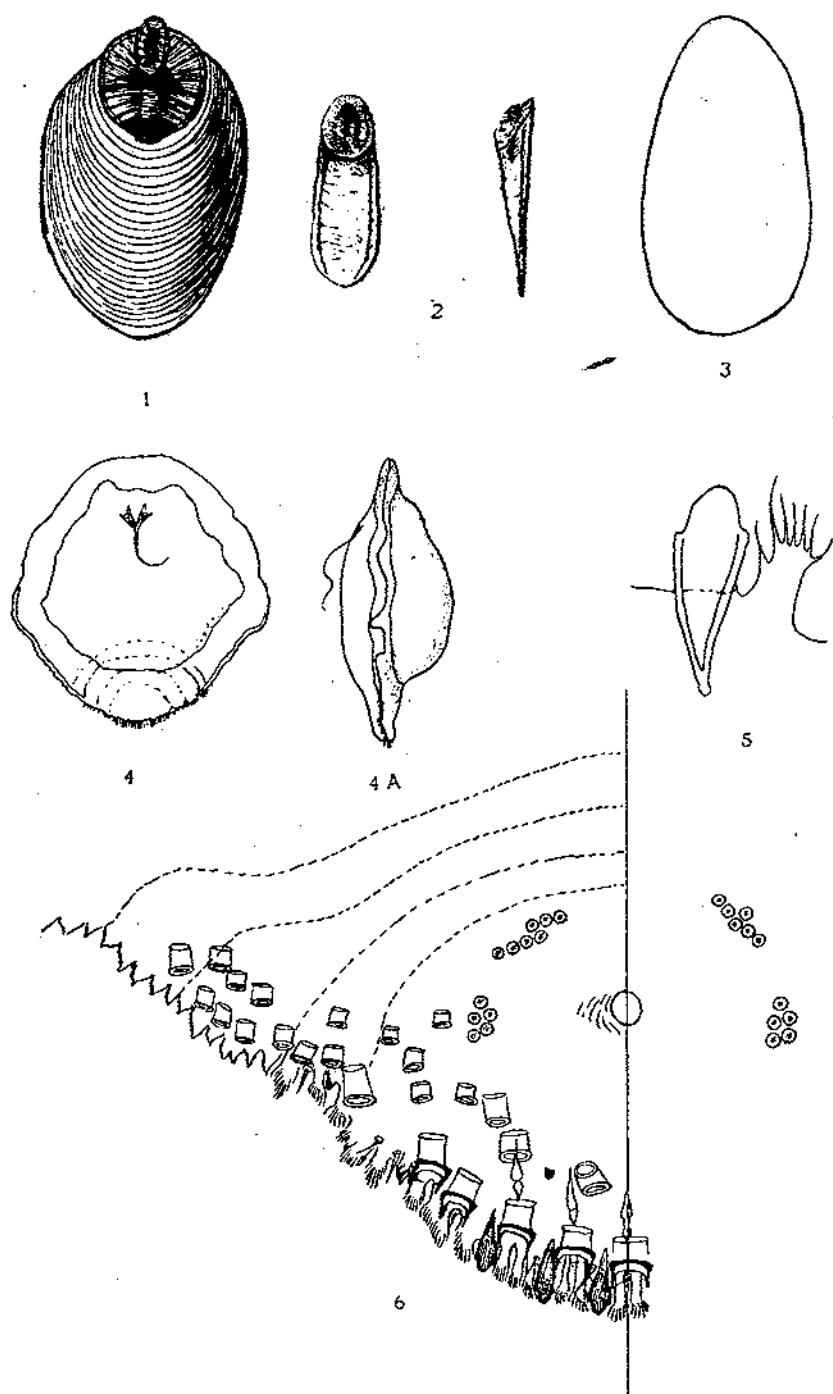


PLATE III.

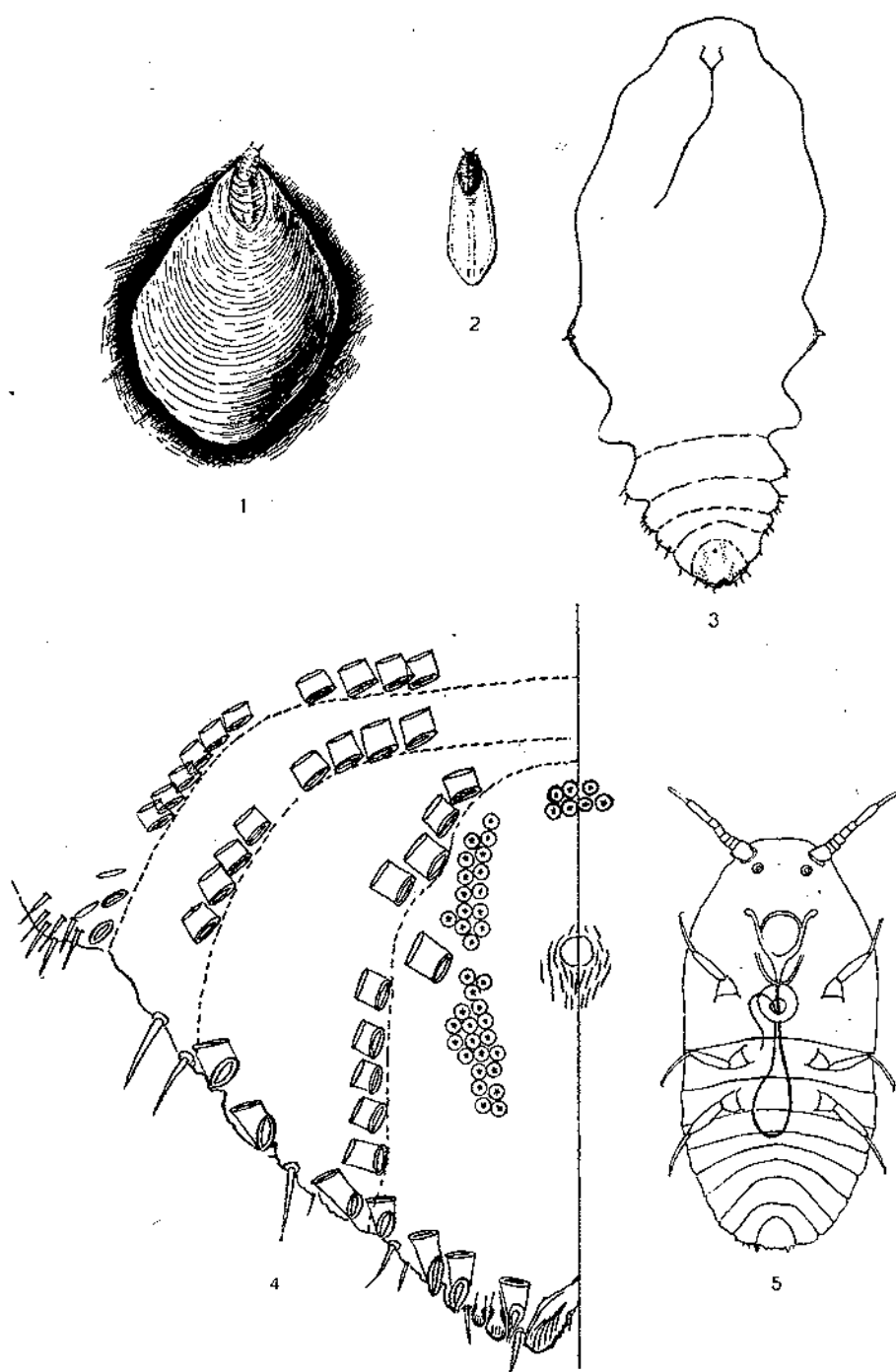


PLATE IV.



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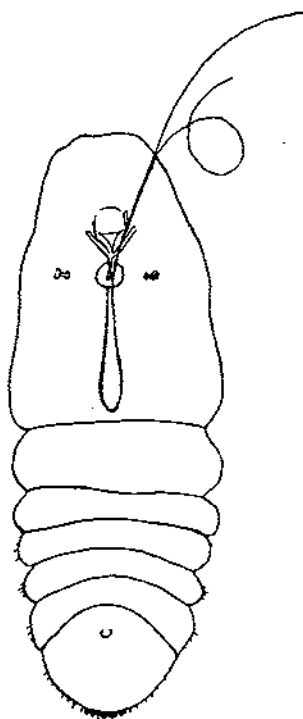
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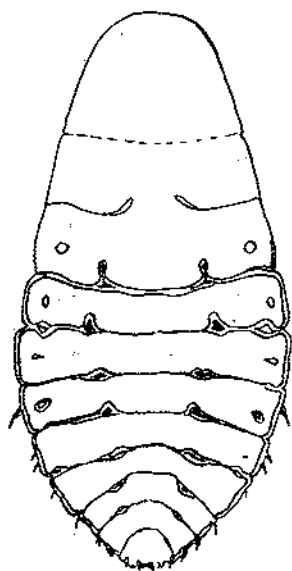
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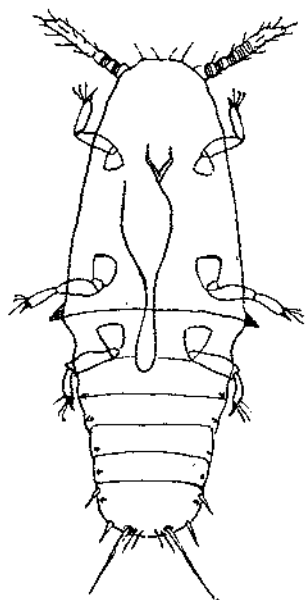
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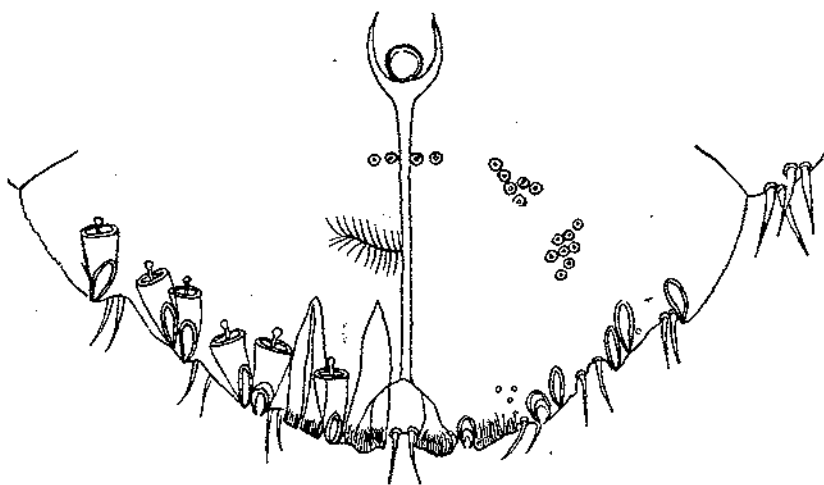
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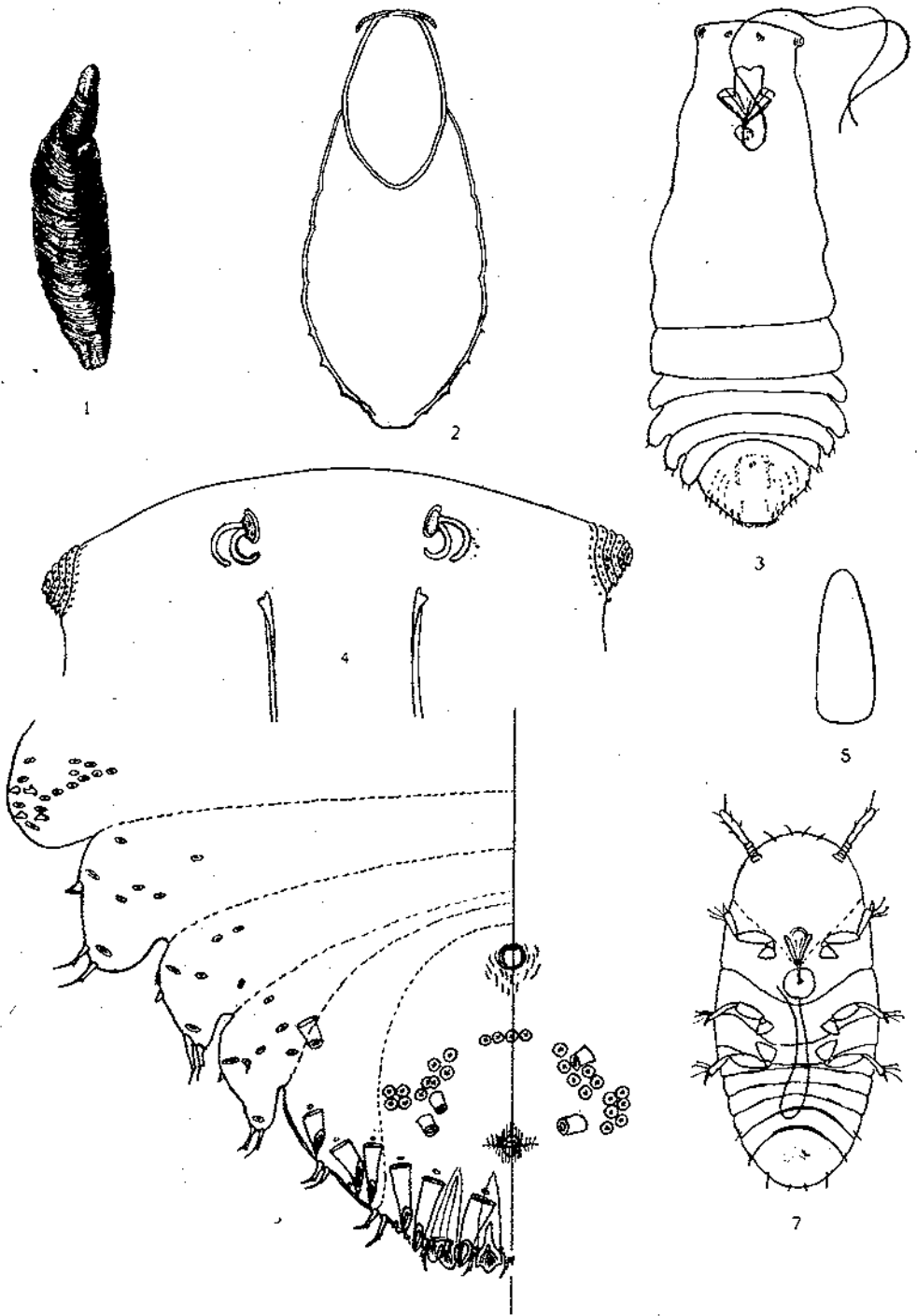


PLATE VII.

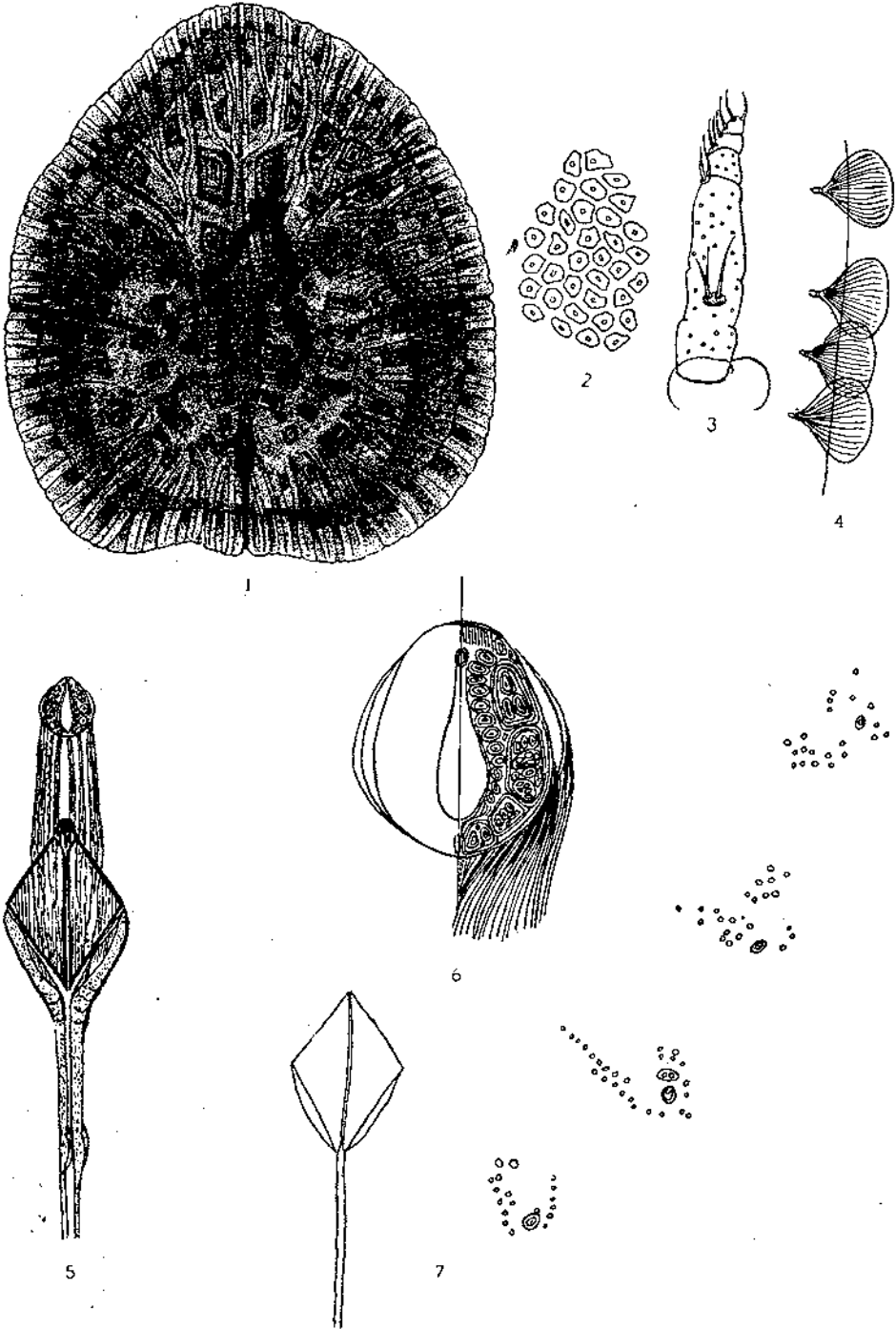
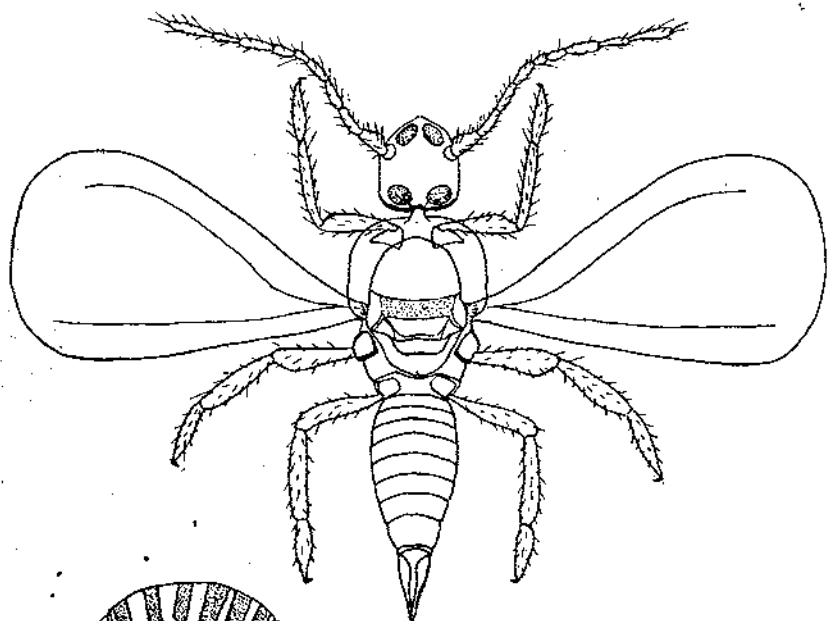
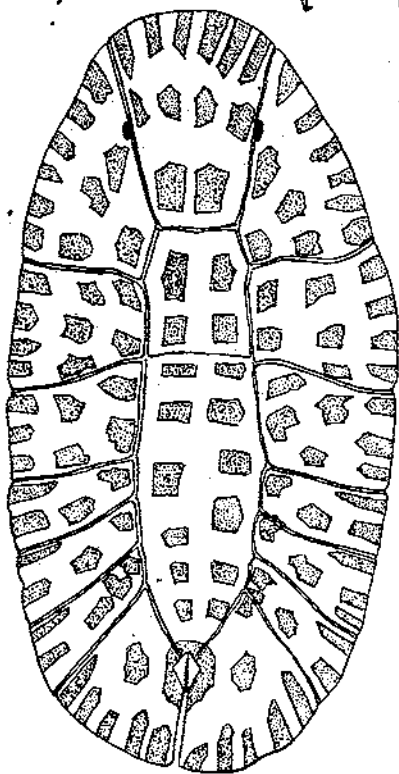


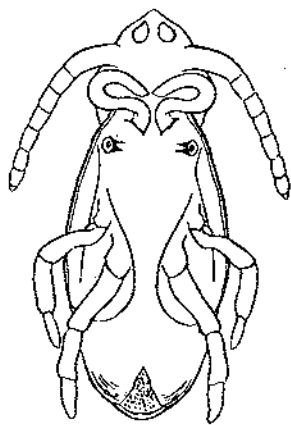
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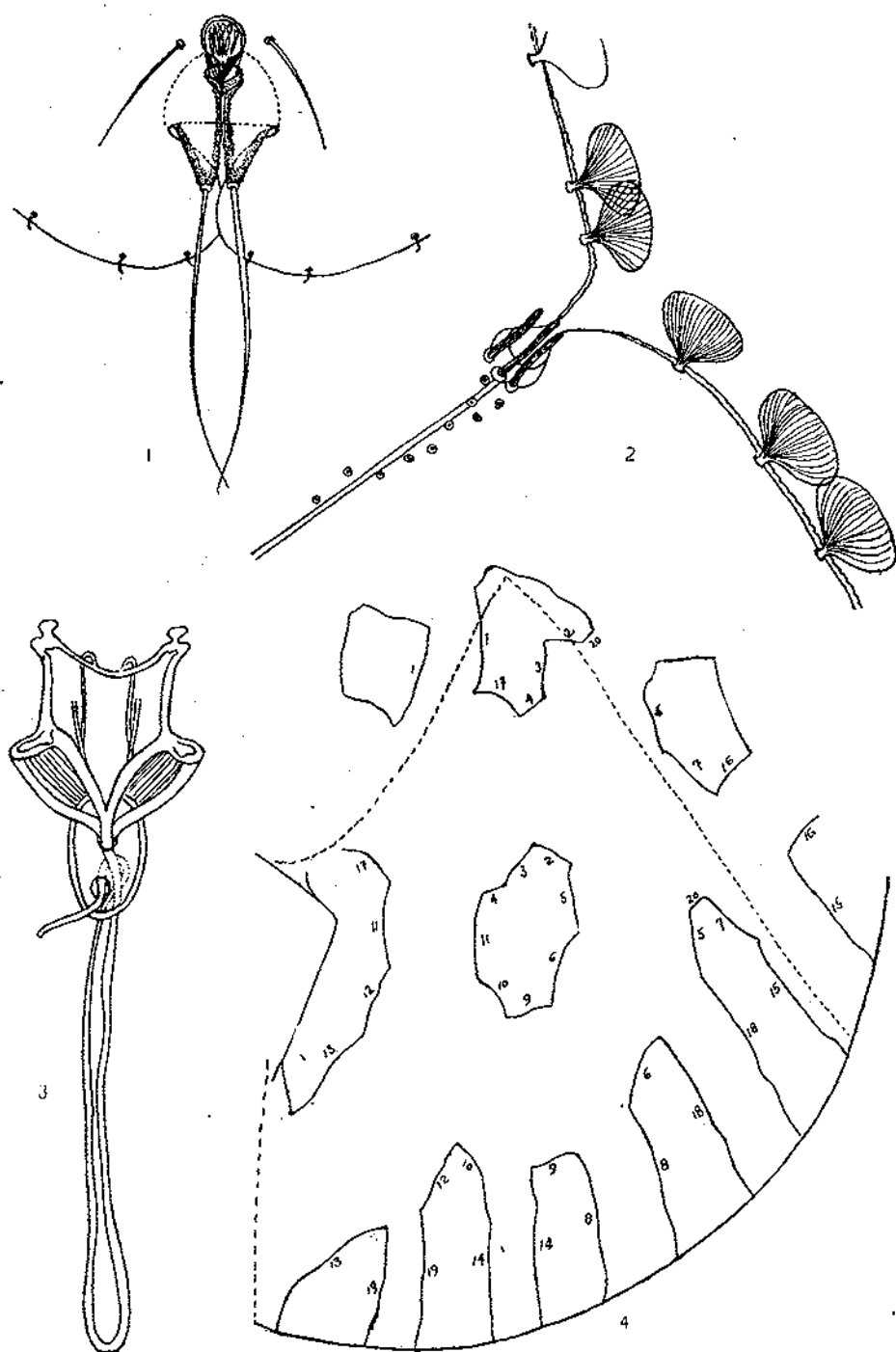


PLATE X.



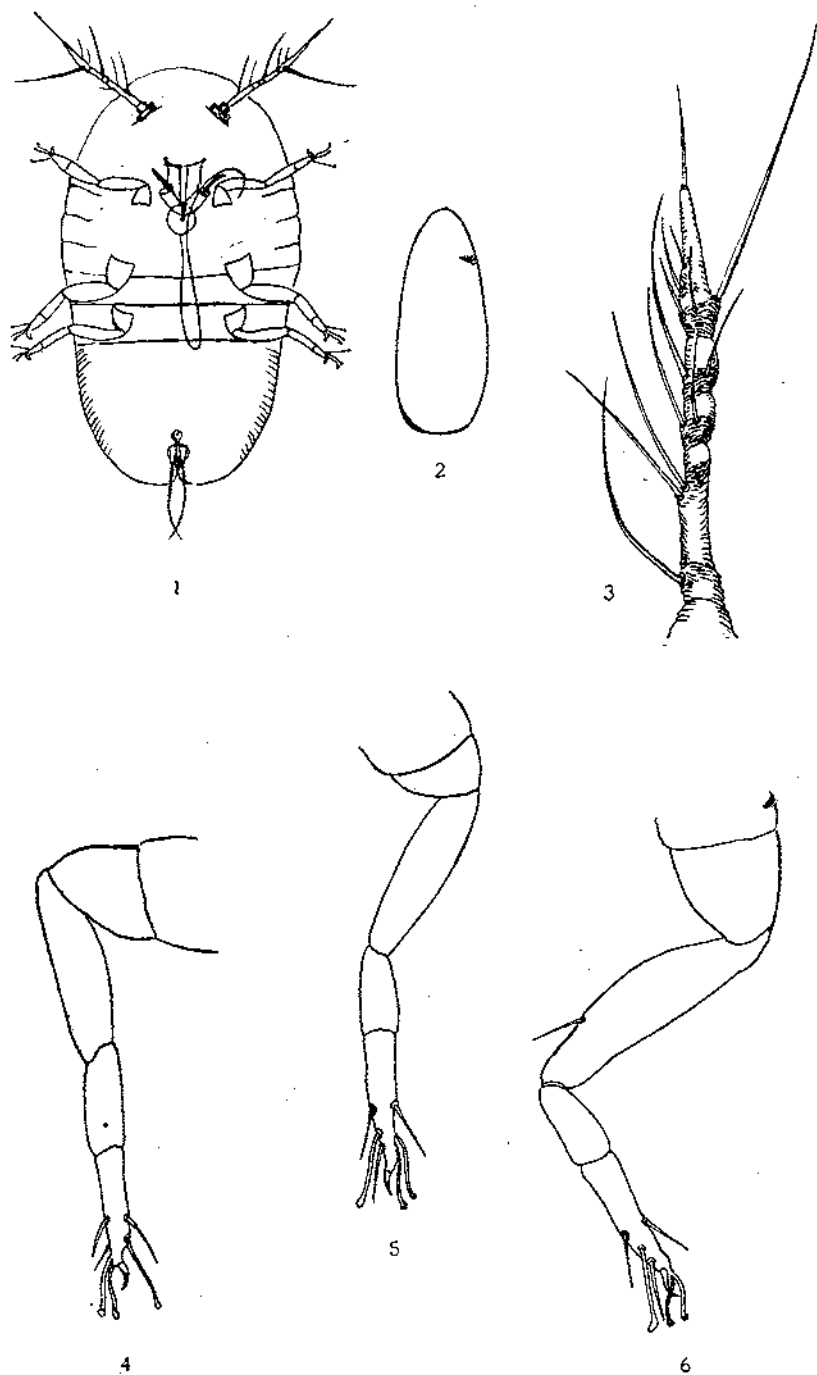


PLATE XI.

# STUDIES UPON EXPERIMENTAL VARIOLA AND VACCINIA IN QUADRUNANA.

By WALTER R. BRINCKERHOFF and E. E. TYZZER, with an introduction by  
W. T. COUNCILMAN.

(From the Sears Laboratory of Pathology, Harvard University Medical School,  
Boston, and the Biological Laboratory, Bureau of Science.<sup>1</sup>)

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<sup>1</sup> Walter R. Brinckerhoff, S. B., M. D., Instructor in Pathology, Harvard University, and Research Fellow of the Rockefeller Institute for Medical Research; E. E. Tyzzer, A. M., M. D., Assistant in Pathology, Harvard University, and Pathologist to the Caroline Brewer Croft Cancer Research, and W. T. Councilman, M. D., Shattuck Professor of Pathological Anatomy of Harvard University.

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## INTRODUCTION.

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The investigation of smallpox is attended with difficulties. The disease only appears at intervals. The cases are not treated in well-organized hospitals where there are facilities for and the habit of the investigation of diseases. As a rule the hospital in which the disease is treated are used only at intervals and are unprovided with laboratories. Clinical teaching, the great stimulus to research, has no place in them. The energies of the physician in charge are entirely taken up in controlling the exigencies of an unusual situation. This isolation of the disease is unfavorable in that the valuable aid given by constant comparison with other diseases is lost.

Difficulties also attend the experimental study of smallpox. Such study can not be carried out in ordinary laboratories, owing to the fear of infection extending from the laboratory.<sup>2</sup> The ordinary laboratory animals such as rabbits, guinea pigs, and all the domestic animals are immune to the disease; the only animal, so far as is known at present, which is susceptible is the monkey. These are expensive, difficult to acquire, and in this climate very susceptible to disease. The majority of monkeys obtained from animal dealers are infected with tuberculosis.

During the epidemic of smallpox which prevailed in Boston in 1901 and 1902, an investigation of the disease was undertaken by members of the pathological department of the Harvard Medical School. The health authorities of the city gave every facility for investigation which was possible. Autopsies were held on 52 cases, embracing all forms of the disease and provision was made enabling certain of the investigators to live in the smallpox hospital and there to undertake some experimental work.

In the course of this investigation it was found that certain cell inclusions, first described by Guarnieri, were constantly associated with the lesions of both vaccinia and variola. These bodies are not of invariable form, but they show a series of forms corresponding to the developmental phases of a living organism. In the course of this development a body much larger and more complicated in structure follows the smallest and simplest forms, which body finally segments into a number of small,

<sup>2</sup> It is remarkable how persistent is this fear of smallpox. That it so persists is an evidence of the horrors of the disease in the prevaccination period. There is no disease which is so feared by the community as is smallpox, and certainly none against which we have such perfect protection.

simple forms corresponding to the ones which are the first to appear in the lesions. In variola, in addition to the forms common to both vaccinia and variola which are found in the cytoplasm of the epithelial cells, a new body appears in the nuclei of the epithelial cells, which undergoes a development dissimilar to that of the cytoplasmic forms, and which finally results in the formation of a sporoblast with spores.

It was believed by the investigators that these inclusions were living parasites and that in both vaccinia and variola there was a simple development taking place in the cytoplasm of the epithelial cells. In smallpox there was a further development which took place within the nucleus and which terminated in the formation of spores, which spores constituted the contagium of smallpox. The material was further worked over by Prof. G. N. Calkins, of Columbia University, who described a life history embracing both the cytoplasmic and intranuclear forms. It was further established that when vaccine virus was inoculated in a susceptible animal (and most animals are susceptible) a typical lesion was produced in which only the cytoplasmic forms of the organism were found. The same was true when variola virus was inoculated in animals not susceptible to variola. No exanthem develops on such animals after inoculation with variola virus. When variola virus was inoculated on the monkey an exanthem analogous to that of variola in man was produced and in both the primary lesion and in the exanthem the cytoplasmic and the intranuclear forms of the parasite were present.

The investigation in Boston was brought to a close by the lack of cases and the difficulties of experimental work. It had been carried to a point where further experimental work was necessary to secure all phases of the disease variola, to study further the interrelation between variola and vaccinia and the immunity problems involved. For these purposes the extensive use of monkeys as experimental animals was indispensable.

To secure experimental material, the best conditions for laboratory work, with access to variola in man to obtain fresh virus, the Biological Laboratory in Manila seemed to offer the best facilities. The proposition was favored by the United States Philippine Commission, who gave us every facility for the prosecution of the work, and Drs. W. R. Brinckerhoff and E. E. Tyzzer, who had taken a prominent part in the investigation of smallpox and vaccinia in Boston, were sent to Manila. To the Hon. Dean C. Worcester, Secretary of the Interior of the Philippine Islands, we wish to express our appreciation and thanks for his numerous efforts in our behalf. Dr. Paul C. Freer, Director of the Bureau of Science,<sup>3</sup> took much interest in our work and essentially furthered it. Dr. Richard P. Strong, Director of the Biological Laboratory, Bureau of Science, in which we worked, was a constant support to us in our work and did much to make

<sup>3</sup> Since the completion of the experimental work detailed in this paper, the Bureau of Government Laboratories of the Philippine Islands has been increased in its scope and its name has been altered to The Bureau of Science.—EDITOR.

our stay agreeable as well as scientifically successful. We wish also to extend our thanks to the members of the laboratory staff, especially to Dr. Wm. E. Musgrave, Pathologist, Dr. Wm. B. Wherry, Bacteriologist, and Dr. Maximilian Herzog, Pathologist, for many favors.

The constant supply of fresh variola virus requisite for our work was secured through the kindness of Maj. E. C. Carter, Commissioner of Public Health, and of Dr. V. G. Heiser, Chief of the Quarantine Service. A constant supply of fresh vaccine virus was given us by Dr. Paul G. Woolley, chief of the Serum Laboratory, Bureau of Science. Dr. W. R. Moulden, resident physician at Bilibid Prison, and Dr. H. B. Wilkinson, resident physician at the San Lazaro Hospital, gave us opportunity to study the clinical material under their control.

The greater part of the funds which made the expedition possible was generously supplied by Mr. Augustus Hemenway, Dr. John C. Phillips, and Dr. Wm. L. Richardson. The Rockefeller Institute for Medical Research aided the expedition by appointing one of the workers to a research fellowship.

If material taken from a smallpox lesion on man be inoculated on an epithelial surface of a calf, after a definite period, a lesion which anatomically closely resembles the parent lesion, the pock is produced. Its appearance is accompanied by swelling of the nearest lymph nodes, fever and constitutional disturbance. After the process has subsided there is immunity to further inoculation. The material from the lesion, transferred to an epithelial surface on another calf, produces a similar result, and after a series of transfers from animal to animal may be returned to man, and it develops not the original disease, smallpox, but the incomparably milder disease, vaccinia.

Many of the strains of vaccine virus now used are known to have been derived from smallpox and we are justified in believing that all strains were originally so derived. Just how many transfers from animal to animal is necessary before the virus loses its power to produce smallpox is not known. One of our experiments in this regard is interesting. The contents of a smallpox vesicle in a monkey was used to inoculate the cornea of a rabbit. After 5 successful transfers to other rabbits the virus was used to inoculate a monkey and not vaccinia, but smallpox was produced. The disease vaccinia confers immunity not only against vaccinia but against smallpox. The immunity, although not absolute, is stronger than is developed by most other infectious diseases. Vaccinia differs from smallpox in three striking respects:

First. The period of incubation is shorter, being in man 5 days. The incubation period of smallpox is 12 days.

Second. In vaccinia there is no general exanthem. There may be a few vesicles around the point of inoculation, but they develop simultaneously with and not after the main lesion and are probably due to a distribution of the virus at the time of inoculation.

Third. For the development of vaccinia it is necessary that the virus directly reach a susceptible epithelial surface. It may be placed on such a surface or it may be carried there by the blood after having been injected into the blood circulation. The disease may also be transferred from individual to individual by immediate or intermediate contact, but there is no evidence that the virus can be transported by the air as can that of smallpox.

It agrees with smallpox in the similarity of the lesion produced by inoculation to the pock and in the fact that both diseases may be produced by the virus of variola.

If material from a smallpox lesion be placed in contact with a susceptible epithelial surface of man or of the monkey, there develops at the site of inoculation a lesion larger, but having the general characteristics of the pock, together with constitutional disturbances and an exanthem less abundant but otherwise similar to the exanthem of smallpox. Immunity to both vaccinia and smallpox follows the disease. All that we know of *variola inoculata* in man is from the old literature. Inoculation of smallpox to confer immunity is no longer practiced in civilized lands. Plehn mentions that it is still practiced among the natives in Central Africa. We know that the disease so produced is incomparably milder than smallpox. The best results were obtained when the inoculation was made superficially. The period of incubation is 8 days. There is no doubt that the practice of inoculating smallpox to confer immunity would have been extensively used and possibly still used were it not for the fact that the inoculated individual is capable of transmitting to others the true disease. There is no qualitative difference in the virus of *variola inoculata* as compared with that of *variola vera*. The disease differs from *variola vera* in its milder course and shorter period of incubation. There are no records of inoculation being made elsewhere than on the skin. The lymph nodes become swollen, but there have been no histological examinations of the skin lesions nor of the internal organs of man.

We believe that the disease which is produced in monkeys by inoculation with smallpox virus most closely corresponds with *variola inoculata* in man and we have so spoken of it. In *variola vera* the infection is due to a virus which can be carried by the air, and infection usually takes place without either mediate or intermediate contact. The monkey is not susceptible to an air borne-virus. The disease was never transmitted from an infected animal to others in the same cage. The monkeys were exposed to the disease in the wards and infected material was placed in the cages with them. Thinking that the anthropoid apes might prove more susceptible, Orang utans were procured from Java and subjected to the same conditions. One of the Orang utans was given a blanket from a smallpox patient, which it used to cover itself with, but without infection ensuing.

The leucocyte reaction in smallpox is striking. In the early skin lesions there is an absence of leucocytes, the blood shows a hypo-leucocytosis, and marrow and spleen show absence of formation of polynuclear leucocytes. There is increased activity in both marrow, lymph nodes, and spleen, but the differentiation of young cells into polynuclear ones does not take place. In the monkey there is none of this. Both the area of inoculation and the exanthem shows an abundance of polynuclear leucocytes, and the marrow shows a leucoblastic activity. It would be most important to know if this were also true of *variola inoculata* in man.

The shortness of the period of incubation in monkeys also speaks in favor of *variola inoculata*, but there is no *variola vera* with which to compare it. The incubation period of vaccinia in man varies as compared with that of animals. The first thought which arises in endeavoring to form an hypothesis in explanation of the difference between *variola vera* and *variola inoculata* in man is that the inoculation is made into a relatively resistant tissue, and before the organisms have time to develop sufficiently and so to infect the blood that an extensive skin eruption is produced by embolism, the organisms are destroyed or rendered inert by the immune substance. This would satisfactorily explain the mild course but not the short incubation period. The incubation period in the monkeys was found to be very definite. It did not materially vary whether the inoculation was made in the trachea, or by blowing dried virus into the lungs, or by injecting it into the blood. In the inoculated monkeys the lesions in the bone marrow and testicle, which we have learned to regard as a characteristic feature of smallpox, are absent. In one monkey only, in which an abundant exanthem followed intratracheal inoculation, a single characteristic lesion was found in a seminal vesicle. We do not know that a virus similar to that which produces the infection in *variola vera* is formed in the monkey. The only way this could be proven would be by exposure of nonprotected individuals.

*Variola inoculata* in the monkey differs from *variola vera* in the relatively smaller numbers of the intranuclear parasites which are present. The same forms are found as in man, but are so few that a prolonged search may be necessary to find them. They were found in greatest numbers in 2 cases, one an Orang utan and the other a Philippine monkey inoculated in the trachea. Of course, we know nothing as to the relative abundance of intranuclear forms in *variola inoculata* in man.

Certain experiments were made in the Philippines with reference to the immunity produced by vaccinia as compared with that produced by *variola inoculata*. These experiments were not sufficiently numerous and varied to cover the entire field. However, they show certain interesting features. The immunity produced by vaccinia is stronger and more fully protective than that produced by *variola inoculata*. Further, vaccinia is a more potent virus than that of variola. It was found easier to produce immunity to *variola inoculata* than to vaccinia. The evidence is that



the immunity is germicidal in character for the serum of an immune animal renders vaccinia virus inactive. We have not been able to make any tests with the serum of monkeys immunized by the variola virus. Experiments made to test the influence of unfavorable external conditions on the virus of vaccinia and variola showed that vaccinia was much more resistant. The variola virus seems to undergo an attenuation after passing through a series of monkeys, finally losing the power of producing an exanthem, although a typical local lesion followed inoculation. The same was true of variola virus which had been subjected to the influence of glycerin for various periods.

It is generally believed that in man the primary variola infection takes place on some mucous surface, systemic infection following from the development of the organisms at the primary focus. There is no anatomical evidence for this assumption. Such a protopustule has never been found. In the 52 autopsies made in Boston careful search was made for such a lesion, but in vain. The period of inoculation in smallpox runs its course without symptoms, but it would be possible for such a lesion to exist in the lungs without producing symptoms, as can a considerable tuberculous focus. We have rarely produced the evidence of systemic infection shown either by immunity or exanthem by inoculating on the mucous membrane of the nose, mouth, or palate, or on the cornea. In these places, owing to the absence of a dense, horny layer which would retain the products in the lesion, an open ulcer was formed and absorption was prevented. Systemic infection did result both from intratracheal inoculation and from blowing the dried virus into the lungs. In this case absorption would take place both from the lesion and from the mucous surface. The type of disease produced in the monkey was not affected by varying the place of inoculation. By insufflating the virus into the lungs a peculiar form of pneumonia was produced with proliferation of the alveolar epithelium and with the cytoplasmic forms of cytoryctes in the epithelial cells. The evidence shows that variola infection can take place in the lungs.

The work in Manila has confirmed what was stated in the earlier publications concerning the cytoryctes, but has added nothing. We feel sure from our work that the inclusions in the cells of the lesions are living organisms. It seems also sure that the organism described does not conform to the type of the other known organisms. The evidence that the things described are living, comes in part from the analogy of structure with other things which are generally recognized as living organisms, and in part from the analogy with living things which they give by progressive growth and differentiation of structure. Certain forms are found at certain intervals of time and they occur in sequence. It has not been possible to show in them nuclear material with the Romanowsky stain nor, so far as I know, has it been possible to stain with this the nuclei of malarial parasites in tissues. In the investigation of smallpox we are

unfortunately limited to the tissues. We have never been able with certainty to detect either in the virus of smallpox or vaccinia, or in the blood of an infected animal or man, the forms which in the tissue we recognize as parasites. If the very minute bodies which we speak of as gemmules in the cytoplasmic cycle and as spores in the nuclear cycle were present in such fluids, we do not know how they could be recognized. The bodies can only be regarded as parasites or as products of cell degeneration. If degenerations, they are totally unlike any of the ordinary substances found in degenerating cells. Moreover, their presence inaugurates the cell changes which are found in the lesions. The cytocytes occur in cells which but for their presence show no departure from the normal type. They are specific. No other disease shows the same changes in the cells. If degenerations, it would be necessary to assume that the virus of smallpox causes certain cells to produce substances within them which have a certain form and size and which grow and change their structure with growth. In other diseases such as *molluscum contagiosum* and in the *epithelioma contagiosum* of fowls we find substances in the cells which are regarded as degenerations and which in their mass and extent are specific. But the character of the degeneration is not specific, and the same changes are found in single cells in other processes. Along with the inclusions in cells, which are definite in size and form and which we recognize as parasites, there are others which are irregular and indefinite. We are inclined to regard these in part as imperfectly developed or degenerated parasites. The immune substance produced in variola and vaccinia has been shown to act as a germicide. It may be formed in the lesion or elsewhere, but it certainly is present and may exert its influence on the organisms which are present. We believe that the inclusions are living organisms for the reason given and that they are the cause of the disease because their relation to the lesions is that of other causal organisms.

In our work on smallpox which is now nearing its temporary conclusions, certain questions have presented themselves. These questions relate: First, to the parasite and its life history. Second, to the inter-relationship of *vaccinia*, *variola inoculata*, and *variola vera*. Third, the immunity and its mode of production. Fourth, to the mode of production of the exanthem. Fifth, to the mode of infection in *variola vera*. In our work both in Boston and Manila we feel that we have made some contributions to all these questions. None of them are completely answered. Their answers involve long and arduous work by skilled investigators, on both vaccinia, variola in man, and the experimental disease in monkeys. For the work a constant supply of virus and of animals for experiment is necessary, and we believe that the work can only be carried out in places where both these conditions can be fulfilled. The most important contribution which could at present be made would be the discovery of an animal in which *variola vera* can be experimentally produced.

## A CRITICAL REVIEW OF THE LITERATURE ON EXPERIMENTAL VARIOLA AND VACCINIA IN THE MONKEY.

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The literature bearing upon the reactions of the monkey to inoculation with the virus of vaccinia or of variola is to be found in fifteen publications, which are briefly summarized below. These articles will first be considered separately in chronological order and the findings will then be combined so as to present a connected account of our present knowledge of the subject:

Zulzer, 1874, attempted to produce variola in "*Cercopithecus*." This author describes briefly the results of experiments on 5 monkeys. Two animals were fed with a mixture of bread and variolous material. No disease followed this procedure. The hair was clipped from an area on the back of one monkey, care being taken not to injure the skin, and variola virus was put in contact with the skin, where it was allowed to remain for three hours. The animal showed no reaction. One monkey was inoculated on the skin with blood from a case of hæmorrhagic variola. This monkey showed a rise in the body temperature which ranged between 40.8° and 41.4° C. from the sixth to the eleventh day of the experiment. A profuse general exanthem developed. One monkey was given dried variola virus to play with and in this animal the same phenomena was seen as in the animal inoculated with the variolous blood.

Buist, 1887, records certain experiments which are only of value in that they showed the susceptibility of the monkey to variola and to vaccinia.

Copeman, 1894, reports a rather extensive series of inoculations of "*Rhesus*" monkeys with variola virus and with 2 sorts of vaccine virus. He found them susceptible to all 3 viruses and also found that each virus protected against a second inoculation with the others. Two monkeys inoculated subcutaneously with vaccine virus were later shown to be immune to skin inoculation with the same virus. One monkey received an intraperitoneal injection of oxalated plasma from a monkey which had been rendered immune to vaccinia and to variola by inoculation. This animal was vaccinated 14 days later and it was noted that the lesions did not develop as well as upon a control monkey inoculated at the same time with the same vaccine virus. This author finds that the acme of the lesion at the site of inoculation is on the eighth day, both when vaccine and when variola virus is used. Vesiculation is not so marked in the pock resulting from variolation as in that following vaccination. A general exanthem was noted in some of the monkeys after inoculation with variola virus. These monkeys also showed constitutional reaction to the inoculation, as was evidenced by fever, diarrhæa, suffusion of the eyes, and some malaise. The temperature reaction was more marked in them than in the monkeys inoculated with vaccine virus.

In 1895 Sternberg reported the results of experiments by Reed in immunizing monkeys against vaccine with the serum of vaccinated calves and monkeys. *Cercopithecus mona*, "*Rhesus*," and *Cobus apella* were employed. In two instances

*C. mona* was shown to react in a typical manner to skin inoculation with vaccine virus. "Rhœsus" also yielded a typical reaction, but in *C. apella* the lesion following vaccination pursued a milder course. The author found that the serum of a vaccinated monkey protected *C. apella* against subsequent vaccination. The serum of the vaccinated calf, even when given in large amounts, only retarded vaccination.

De Haan, 1896, reported upon an extensive series of inoculations of *Macacus cynomologus* with vaccine, retrovaccine, and variola virus. Forty-three monkeys were used in this research. This author found *M. cynomologus* to be susceptible to all 3 of the viruses used. Only a local lesion followed inoculation with vaccine, while a general exanthem was observed in 25 per cent of the monkeys inoculated with variola virus. Both retrovaccine and vaccine protected this species of monkey against subsequent inoculation with variola virus. The acme of the vaccine lesion in this monkey was reached on the seventh day, as in man, while in the calf the lesion was at its height on the fifth day. Vaccine, retrovaccine, and "la variole mitigée" tended to die out if transferred too long on one species. A strain of vaccine virus which became attenuated was reactivated by transferring it to another species and then inoculating it back on the original species. Vaccination of the skin of the monkey protected the animal against subsequent inoculation of the skin with vaccine virus. A strain of vaccine virus gave typical lesions for seven passages through the monkey. The series of animals so inoculated showed the same immunity to subsequent inoculation with vaccine virus. Monkeys successfully inoculated on the skin with vaccine virus were immune to subsequent inoculation of the skin with variola virus. A strain of variola virus was carried from one monkey to another for seven passages, in each case yielding good primary lesions but no general exanthem. After 6 or 7 such passages the strain was inoculated on the calf and produced a vaccine-like lesion. The lesion rendered the calf immune to subsequent inoculation with vaccine virus.

Reed, 1897, found peculiar bodies in the blood of vaccinated and variolated monkeys, but the various controls showed similar bodies and he was not able to produce evidence either of their specificity or of their parasitic nature.

Béclère, Chambon, and Menard, 1899, inoculated three "Macacus" monkeys with variola virus. Primary lesions developed at the site of inoculation which were identical with those following inoculation with vaccine virus. The monkeys showed a constitutional reaction evidenced by diarrhœa, fever, œdema, and albuminuria, and died on the fourteenth day. The serum of such a monkey was shown to have antivirulent properties when put in contact with vaccine virus.

Roger and Weil, 1902, produced lesions by inoculating "Macacus" monkeys with variolous material. Two animals which were inoculated on the skin with variola virus developed typical lesions at the site of inoculation. These monkeys were subsequently shown to have acquired immunity to a second inoculation of the skin with variola virus. One monkey was inoculated on the skin with blood from a case of hæmorrhagic variola. A few small pustules developed at the site of inoculation. Four monkeys inoculated subsequently with blood from a case of hæmorrhagic variola showed no specific lesions. One of these animals died of septicæmia. The monkeys inoculated on the skin with variola virus and the one inoculated on the skin with variolous blood yielded a positive, though imperfect, reaction, when subsequently inoculated on the skin with vaccine virus. Two of the monkeys which had been inoculated subcutaneously with variolous blood were immune to skin inoculation with vaccine, while in one an abortive lesion resulted.

Ewing, 1902, inoculated a "Rhœsus" and a "large African monkey" with variola virus. Both animals gave a positive reaction to the inoculation. He also inoculated monkeys with bacteria of various sorts which had been isolated from cases of variola, always with negative results.

Park, 1902, inoculated 6 monkeys on the skin with variola virus. All the animals yielded a typical pock at the site of inoculation. Both fresh and dried virus was found active on the monkey. He found monkeys refractory to inoculation with virus from cases of varicella and points out the value of such a test in diagnosis of obscure cases where a differential diagnosis must be made between variola and varicella. This author considers "Java" monkeys most suitable for the test.

Blaxall and Fremlin, 1903, show that by feeding monkeys with vaccine virus mixed with their food, specific lesions may result in the mouth (one case), or the mesenteric lymph nodes (one case) may become a locus of the virus. Their results were controlled by inoculations on the calf.

Magrath and Brinckerhoff, 1904, found "Macacus" and "Rhesus" monkeys to be susceptible to inoculation with variola virus. They call attention to the fact that the disease produced is related to *variola inoculata* in man rather than to *variola vera* and that the specific lesions produced in these monkeys contain *Cytoryctes variolæ*. In a second paper the minute study of the specific lesions is presented and it is shown that both the cytoplasmic and the nuclear forms of the parasite are present.

Magrath and Brinckerhoff, 1903, in studying the blood of variolated and normal monkeys found a variety of bodies which they interpreted as derivatives of the blood cells of the animal.

The difficulty of getting monkeys in those places where the bulk of scientific work is carried on explains the scanty data to be found in the literature upon the reactions of this animal in variola and vaccinia. As the foregoing summary shows, the only worker who had animals enough to do adequate controls and to inoculate a sufficient number for the solution of his problems was De Haan, who worked in Java, where monkeys were abundant. In spite of the comparatively small number of experiments recorded by other investigators, it seems worth while to combine the data in the following summary.

That the monkey is susceptible to vaccinia or to variola has been the experience of all students of the subject. In some cases the species of monkey used is known, and we find that *Macacus cynomologus* is susceptible to variola and to vaccinia (De Haan), *Cercopithecus mona* and *Cebus apella* are susceptible to vaccinia (Reed). "Rhesus" monkeys were successfully inoculated with vaccine virus by Copeman and by Reed, and with variola virus by Copeman, by Ewing, and by Magrath and Brinckerhoff.

"Macacus" monkeys were found susceptible to variola virus by Bédère, Chambon, and Menard, by Roger and Weil, and by Magrath and Brinckerhoff. An "African" monkey (Ewing), "Java" monkeys (Park), and a "Cercopithecus" (Zulzer) have also been shown to react to inoculation with variola virus. It is unfortunate that the species of monkey used by various investigators is not known, as it may be possible, from statements made in the literature, that different species of monkeys may present different degrees of susceptibility both to the contagium of variola and to that of vaccinia.

The acme of the process at the site of inoculation in vaccination is placed by Copeman at the eighth day of the disease. De Haan finds it

at its height on the seventh day. Copeman notes that the vesiculation is more marked in vaccination than in variolation. Reed finds that *Cebus apella* presents vaccine lesions which run a milder course than they do in the other monkeys used by him. Several investigators compare the lesion produced in the monkey by inoculation of the skin with vaccine virus, with that which develops in the calf and speak of them as being identical.

The constitutional reaction of the monkey to vaccination is mentioned by Copeman and said to be less intense than that following variolation, at least so far as is evidenced by the body temperature.

A general exanthem has never been observed to follow inoculation of the skin of the monkey with vaccine virus.

The immunity reactions of the monkey after vaccination have received considerable attention. Copeman reports that vaccination of the skin of the "Rhesus" monkey protects against subsequent inoculation of the skin with either vaccine or variola virus. De Haan found the same protection to be conferred upon *Macacus cynomologus* by vaccination.

The protective power of the serum of a vaccinated animal has been tested by several workers. Copeman reports that the oxalated plasma of a monkey, immune to vaccination and variolation of the skin through inoculation, has a slightly modifying effect upon the course of a vaccination in a monkey into whose peritoneal cavity the plasma has been introduced. He also finds that the monkey is rendered immune to vaccination by the subcutaneous inoculation of vaccine virus. Reed finds that the serum of a vaccinated monkey protects *Cebus apella* against subsequent skin inoculation with vaccine virus. The serum of vaccinated calves was not so efficacious in this respect.

Certain miscellaneous experiments are of interest. De Haan finds that vaccine virus tends to die out if transferred too often on one species of animal. The fact that he worked in the Tropics may have influenced his results. Blaxall and Fremlin show that vaccine virus in the food of a monkey may produce lesions in the mouth or may enter the mesenteric lymph nodes and be demonstrable there by inoculation of an emulsion of the nodes upon the skin of the calf.

Inoculations of the monkey with variola virus have been undertaken by a number of observers. In all cases the monkey has been shown to be susceptible to the contagium.

The contents of the specific skin lesion of variola in man has been used for inoculation by Zulzer, Copeman, De Haan, Bécélère, Chambon, and Menard, Roger and Weil, Ewing, Park, and by Magrath and Brinckerhoff. As a rule the virus was used in the fresh state, but Zulzer and Park have both employed dried virus as well. The blood from a case of hæmorrhagic variola was used by Zulzer and by Roger and Weil.

Inoculation of a scratch or scarification on the skin has been used as the mode of introducing the contagium in a majority of the experiments. Simple contact with the unbroken skin as well as exposure to dried and

finely divided virus was tried by Zulzer. The subcutaneous injection of blood from a case of hæmorrhagic variola was done by Roger and Weil.

The data on the constitutional reaction of the inoculated animal are rather scanty. Fever has been noted by Zulzer, by Copeman, by Bécélère, Chambon, and Menard, and by Magrath and Brinckerhoff. Diarrhœa was observed by Copeman and by Bécélère, Chambon, and Menard. Three investigators report animals dying after inoculation of the skin with variola virus. In the case of Bécélère's animals it seems evident that there was intercurrent disease. The animals inoculated with fatal results by Roger and Weil and by Magrath and Brinckerhoff were shown to have died either from streptococcus septicæmia or from tuberculosis.

In all experiments where the skin was inoculated, a pock which closely simulated that produced by vaccination was observed to develop at the site of inoculation.

A general exanthem was observed by Zulzer, Copeman, De Haan, and by Magrath and Brinckerhoff. The virus seemed to have lost this power of producing an exanthem when transferred from one monkey to another (De Haan).

The immunity developed by inoculation with variola virus has been studied by Copeman and by Roger and Weil. The former found that variolation of the monkey protected against subsequent inoculation with vaccine or variola virus. The latter found that inoculation of the skin with variola virus did not confer complete immunity to later inoculation with vaccine, although the animals were immune to variolation by skin inoculation.

That the serum of a variolated monkey had "anti-virulent" properties when put in contact with vaccine was shown by Bécélère, Chambon, and Menard.

It was shown by De Haan that a strain of variola virus which had been passed from one monkey to another for six or seven generations was *inoculable* on the calf.

A careful analysis of the evidence in the literature seems to show that the disease produced in monkeys by various inoculations with variola virus has always been one which conforms closely to the type of *variola indculata* as seen in the human subject. This was emphasized by Magrath and Brinckerhoff.

We find no reference to the occurrence of smallpox on monkeys in the wild state among the species inhabiting the Old World. The statement is sometimes made that monkeys in the Western Hemisphere suffer from epidemics of the disease in localities where smallpox is epidemic among men. This statement seems to be based upon the following data.

Andrew Anderson, in his book on Fevers, gives an excerpt from a letter that he received from a friend who was traveling in Central America. This statement has frequently been referred to and it seems worth while here to give it in full.

In the year 1841 I was in the Province of Veragua, in New Granada, to the north of the Isthmus of Panama and left the town of St. Jago on the western coast for David in Chiriqui, a town in the interior, about 60 or 70 miles to the northeast (and leeward) of St. Jago. The smallpox was raging with great violence in St. Jago, but there was no appearance of it in David. A few days after my arrival there, taking my customary morning's ride in the forest, which teems with animal life, I was struck by observing one or two sick and apparently dying monkeys on the ground under the trees. The next morning I was struck by the same singular appearance (for it is very unusual to find a wild animal sick—they instinctively hide themselves) and by thinking that I perceived several on the trees, moping and moving about in a sickly manner, I consequently dismounted and carefully examined two, which were on the ground—one dead and the other apparently dying; and after careful examination, no doubt remained in my mind that they were suffering and had died from smallpox. They presented every evidence of the disease, the pustules were perfectly formed, and in one instance (that of the dying one) the animal was nearly quite blind from the effects. A few days afterwards (I think about four or five days) the first case of smallpox appeared amongst the inhabitants of David, and in the course of a fortnight one-half of the population was stricken.

In 1858 Dr. Furlong, taking part in a discussion upon diseases in animals, stated that he had received a letter from the wife of a prominent physician of the Island of Trinidad who mentioned that during epidemics of smallpox in that island the wild monkeys suffered from the disease.

Charles Kingsley makes the same statement with regard to an epidemic of smallpox which visited the same island in 1739. We have not been able to find where this author got the information.

In view of the statements cited above one can not deny that the monkey may contract smallpox from man. It is probable that different species of monkeys show different degrees of susceptibility to the contagium. The New World monkeys differ in many respects from their relatives in the Old World and it is quite possible that they are more susceptible to smallpox. The monkeys which are generally used in experimental work in smallpox come from the Eastern Hemisphere. We believe that notable increase in our knowledge of smallpox waits on the finding of an experimental animal in which *variola vera* can be produced. The susceptibility of the New World monkeys in this respect should be tested.

#### BIBLIOGRAPHY.

- FURLONG. Diseases of Animals. *The Veterinarian*, London (1858), XXXI, 142.  
 ANDERSON, A. Study of Fever, London (1861).  
 KINGSLEY, C. At Last: A Christmas in the West Indies. Tauchnitz Edition, (1871), I, 127.  
 ZULZER, W. Zur Aetiologie der Variola. *Cent. f. d. Med. Wiss.* (1874), 82.  
 BRUIST, JOHN. Vaccinia and Variola: A Study of Their Life History. London (1887), Churchill.  
 COPEMAN, S. M. Variola and Vaccinia; Their manifestations and interrelations in the Lower Animals. A comparative study. *Jour. Path. and Bact.* (1894), II, 407.  
 STERNBERG, G. M., and W. REED. Report on immunity against Vaccination conferred upon the Monkey by the use of the Serum of the Inoculated Calf and Monkey. *Trans. Assoc. Am. Phys.* (1895), X, 57.



- DE HAAN, L. J. E. Vaccine et Retrovaccine a Batavia. *Ann. de l'Inst. Pasteur.* (1896), X, 169.
- REED, W. On the appearance of certain amœboid bodies in the blood of vaccinated monkeys (Rhesus) and children, and in the blood from cases of Variola. *Jour. Exp. Med.* (1897), II, 515.
- BÉCLÈRE, CHAMBON, and MÉNARD. Etudes sur l'immunité Vaccinale. *Anat. Inst. Past.* (1899), XIII, 81-125.
- ROGER, H., et E. WEIL. Inoculation au Singe. *Compt. rendu Soc. Biol. Paris* (1902), 1271.
- EWING, J. Preliminary Report of Study of Smallpox. *Trans. Asso. Am. Phys.* (1902), XVII, 263.
- PARK, W. H. A practical method for differentiating variola and varicella by means of inoculation of Monkeys. *Trans. Assoc. Am. Phys.* (1902), XVII, 217.
- BLAXALL, F. R., and H. S. FREMLIN. Experiments in immunizing monkeys by adding vaccinous material to their food. *Rep. Loc. Gov. Board, London* (1902-3), 650.
- MAGRATH, G. B., and W. R. BRINCKERHOFF. On the Occurrence of *Cytoryctes Variolæ*, Guarnieri in the Skin of the Monkey Inoculated with Variola Virus. *Jour. Med. Research* (1904) XI, 173.
- On experimental Variola in the Monkey. *Ibid.*, 230.
- The Infectiousness of the Blood in Variola. *Ibid.*, 267.

## PART I.

# STUDIES UPON EXPERIMENTAL VACCINIA IN THE PHILIPPINE MONKEY (*MACACUS CYNOMOLOGUS*).

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### 1. VACCINIA FOLLOWING INOCULATION OF THE SKIN OF THE MONKEY.

*Technique.*—Different strains of vaccine virus were employed. For convenience the different viruses will be indicated by Arabic numerals. The source of each strain of virus is as follows.

*Virus 1.*—Supplied by the Serum Laboratory of the Bureau of Government Laboratories,<sup>4</sup> Manila. A description of the method of its preparation will be found in the Third Annual Report of the Superintendent of Government Laboratories.

*Virus 148.*—From the New York Board of Health. The pulp was mixed with 60 per cent glycerin.

*Virus 246.*—From the Japanese Imperial Board of Health. The pulp was mixed with 60 per cent glycerin which contained 1 per cent carbolic acid.

*Virus 236.*—From the Vaccine Laboratory of Park, Davis & Co. The pulp was mixed with 60 per cent glycerin.

*Virus 251.*—From the Laboratory of Chambon of Paris. The pulp was mixed with 60 per cent glycerin containing 1.5 per cent carbolic acid.

We are indebted to Dr. J. J. Kinyoun, of Glenolden, Pennsylvania, for the last 4 of these viruses. He sent us a quantity of each of these strains and so arranged matters that, through the courtesy of the steamship company and of the United States Marine-Hospital Service, the virus was kept on ice from the time it left his hands until it reached us in Manila.<sup>5</sup>

The skin of the abdomen was chosen as the site of inoculation. The hair was first shaved from a large area and the skin then scrubbed with soap and water, followed by alcohol. We have found the most satisfactory results to follow the inoculation of a shallow incision. As a rule a number of such scratches were inoculated in each animal. When inoculation is done in this way, the study of the development of the lesion can be made to greater advantage than when scarification is practiced; for the lesion produced by the inoculation instrument is so slight that it heals

<sup>4</sup> Now Bureau of Science.

<sup>5</sup> We take this opportunity of expressing our gratitude to Dr. Kinyoun for his kindness in sending us these strains of virus and to those who assisted in forwarding them.

before the specific process, due to the introduction of the virus, has become visible to the naked eye. The vaccine lesion therefore develops in a skin practically normal and its evolution can better be studied than when the phenomena of repair of a scarification complicates the picture. A general anæsthetic was employed whenever the operation might cause discomfort to the animal.

Daily observations were recorded upon the constitutional reaction of the animal, the temperature reaction per rectum, the reaction of the lymph nodes, and the macroscopic appearances of the specific lesion.

The material for histological study of the process in the skin was collected by excision of lesions. In many instances the animals were killed at various times after the inoculation. In these cases a complete autopsy was performed and the material saved both from the specific lesions and from the viscera. All tissues were put in Zenker's Fluid for 24 hours, then washed in running water for 24 hours, and hardened by passage through graded alcohols. Material for histological study was embedded by the chloroform-paraffin method. Sections were stained in various ways.

#### EXPERIMENTS IN DETAIL.

The experiments on which this article is based comprise the inoculation with vaccine virus of 28 monkeys on the skin of the abdomen. The following experiments are selected to be given in detail.

##### I. *Clinical course of the disease.*

No. 91. Adult male *M. cynomolgus*. Inoculated in 12 places on the abdomen with vaccine virus No. 251. Body temperature 38°.5 C.

Twenty-four hours after inoculation 2 scratches show slight elevation, others show no reaction. Body temperature 38°.2 C.

Forty-eight hours. There is slight elevation and some opacity along the line of inoculation. The axillary lymph nodes are of normal size. Body temperature 39° C.

Three days. There is a distinct elevation with opacity and redness about the narrow crust which marks the line of inoculation. The axillary lymph nodes are slightly enlarged. The body temperature is 39° C.

Four days. A distinct pink elevation is present, extending from 2 to 3 millimeters on either side of the central crust. One lesion appears to be vesicular. Axillary lymph nodes distinctly enlarged. Body temperature 39°.5 C.

Five days. The lesions present as rounded elevations from 4 to 5 millimeters across. The central crust is surrounded by a narrow translucent vesicle which in turn is bordered by a pink areola. Axillary lymph nodes markedly enlarged. Body temperature 40°.2 C.

Six days. The lesions show the same features; but the extent is greater and the whole lesion more sharply circumscribed. Axillary lymph nodes enlarged. Body temperature 39°.5 C.

Seven days. The lesions present a central yellowish crust which is bordered by a vesicle, translucent peripherally but opaque about the crust. The lesions are surrounded by a distinct red areola. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Eight days. The lesions show a slight increase in the extent of the central crust. The vesicular zone has become entirely opaque in places. The axillary lymph nodes are much enlarged. On the right side they form a mass 2 centimeters in diameter. Body temperature 40° C.

Nine days. The lesions present as flat-topped elevations consisting of a peripheral vesicular ring which has become completely opaque, surrounding a macerated area where the crust has been picked off. The whole lesion is surrounded by a pink flush. Axillary lymph nodes enlarged but not so hard as before. Body temperature 39° 4 C.

Ten days. The lesions show an elevated pink, somewhat ragged epithelial edge which surrounds an area which is in part crusted and in part ulcer-like. Axillary lymph nodes somewhat enlarged. Body temperature 39° C.

From this time on the lesions simply showed the phenomena of repair and healed after a few days.

Twenty-three days after the first vaccination the animal was again inoculated on the skin of the abdomen with vaccine virus No. 148. No reaction followed this inoculation.

No. 96. Adult male *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 236. Body temperature 39° 6 C.

Twenty-four hours after inoculation the scratches show considerable elevation with some reddening of the surrounding skin but with no opacity. Body temperature 39° 2 C.

Forty-eight hours. There is slight elevation and some opacity along the line of inoculation. Axillary lymph nodes slightly enlarged. Body temperature 38° 5 C.

Three days. There is an irregular elevation with opacity beside the narrow crust which marks the line of inoculation. Axillary lymph nodes are distinctly enlarged. Body temperature 39° C.

Four days. The elevation which borders the crust has increased somewhat, extending from 2 to 3 millimeters on each side. Axillary lymph nodes much enlarged. Body temperature 39° C.

Five days. Around the crust is a narrow vesicular ring which is bordered peripherally by elevated and reddened epithelium. The lesion is now from 6 to 8 millimeters wide. Axillary lymph nodes large and hard. Body temperature 39° 8 C.

Six days. The lesion presents as a sharply circumscribed elevation consisting of a depressed central yellow crust bordered by an elevated ring from 2 to 3 millimeters broad which is opaque and vesicular near the crust and which is pink externally. Axillary lymph nodes enlarged, some measuring 1 centimeter in diameter. Body temperature 39° 2 C.

Seven days. The lesion is the same as yesterday save that the crust has extended and encroached upon the vesicular ring. Axillary lymph nodes enlarged and hard. Body temperature 38° 8 C.

Eight days. The animal has picked off the crusts and the lesion presents a central excoriated area surrounded by the more or less ruptured vesicular ring. There is a distinct reddening of the skin about the lesion. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Nine days. The crust has formed again in the center of the lesion and about this is to be made out the remnants of the vesicular ring. The areola persists. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Ten days. From this time on the lesions have lost their specific character and simply present the phenomena of healing. The axillary lymph nodes show a diminishing tumor and consistency but are harder than normal for a considerable time after the healing of the skin lesion.

Eight days. The lesions show a slight increase in the extent of the central crust. The vesicular zone has become entirely opaque in places. The axillary lymph nodes are much enlarged. On the right side they form a mass 2 centimeters in diameter. Body temperature 40° C.

Nine days. The lesions present as flat-topped elevations consisting of a peripheral vesicular ring which has become completely opaque, surrounding a macerated area where the crust has been picked off. The whole lesion is surrounded by a pink flush. Axillary lymph nodes enlarged but not so hard as before. Body temperature 39° 4 C.

Ten days. The lesions show an elevated pink, somewhat ragged epithelial edge which surrounds an area which is in part crusted and in part ulcer-like. Axillary lymph nodes somewhat enlarged. Body temperature 39° C.

From this time on the lesions simply showed the phenomena of repair and healed after a few days.

Twenty-three days after the first vaccination the animal was again inoculated on the skin of the abdomen with vaccine virus No. 148. No reaction followed this inoculation.

No. 96. Adult male *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 236. Body temperature 39° 6 C.

Twenty-four hours after inoculation the scratches show considerable elevation with some reddening of the surrounding skin but with no opacity. Body temperature 39° 2 C.

Forty-eight hours. There is slight elevation and some opacity along the line of inoculation. Axillary lymph nodes slightly enlarged. Body temperature 38° 5 C.

Three days. There is an irregular elevation with opacity beside the narrow crust which marks the line of inoculation. Axillary lymph nodes are distinctly enlarged. Body temperature 39° C.

Four days. The elevation which borders the crust has increased somewhat, extending from 2 to 3 millimeters on each side. Axillary lymph nodes much enlarged. Body temperature 39° C.

Five days. Around the crust is a narrow vesicular ring which is bordered peripherally by elevated and reddened epithelium. The lesion is now from 6 to 8 millimeters wide. Axillary lymph nodes large and hard. Body temperature 39° 8 C.

Six days. The lesion presents as a sharply circumscribed elevation consisting of a depressed central yellow crust bordered by an elevated ring from 2 to 3 millimeters broad which is opaque and vesicular near the crust and which is pink externally. Axillary lymph nodes enlarged, some measuring 1 centimeter in diameter. Body temperature 39° 2 C.

Seven days. The lesion is the same as yesterday save that the crust has extended and encroached upon the vesicular ring. Axillary lymph nodes enlarged and hard. Body temperature 38° 8 C.

Eight days. The animal has picked off the crusts and the lesion presents a central excoriated area surrounded by the more or less ruptured vesicular ring. There is a distinct reddening of the skin about the lesion. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Nine days. The crust has formed again in the center of the lesion and about this is to be made out the remnants of the vesicular ring. The areola persists. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Ten days. From this time on the lesions have lost their specific character and simply present the phenomena of healing. The axillary lymph nodes show a diminishing tumor and consistency but are harder than normal for a considerable time after the healing of the skin lesion.

Twenty-three days after the original vaccination the animal was again inoculated on the skin of the abdomen with vaccine virus No. 148. No reaction followed this second inoculation.

No. 98. Adult male *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 1, Lot No. 365. Body temperature 39° 4 C.

Twenty-four hours after inoculation the scratches show slight elevation and opacity. Axillary lymph nodes normal. Body temperature 39° C.

Forty-eight hours. About the inoculation the skin is slightly elevated and opaque. Axillary lymph nodes normal. Body temperature 39° C.

Three days. There is some reddening and marked elevation and opacity of the skin which borders the narrow crust which marks the line of inoculation. Axillary lymph nodes distinctly enlarged. Body temperature 39° C.

Four days. The central crust has increased in extent and is surrounded by a pink elevation from 2 to 3 millimeters broad. Axillary lymph nodes enlarged. Body temperature 38° 4 C.

Five days. The skin immediately about the central crust is slightly translucent, suggesting beginning vesicle formation. Axillary lymph nodes enlarged and hard. Body temperature 39° 5 C.

Six days. The lesion presents a depressed yellow-brown crust which is surrounded by an elevated ring which is sharply circumscribed and is translucent near the crust. Axillary lymph nodes enlarged and hard. Body temperature 39° C.

Seven days. The vesicular ring about the central crust is now more or less opaque. There is distinct areola. Axillary lymph nodes enlarged and hard. Body temperature 39° 8 C.

Eight days. Lesions present same features as yesterday save that there is some induration of the subcutaneous tissue beneath the lesions. Axillary lymph nodes very much enlarged. Body temperature 40° 2 C.

Nine days. The central crust has begun to encroach upon the vesicular ring and the areola is less marked. Some degree of subcutaneous oedema persists. Axillary lymph nodes enlarged and hard. Body temperature 40° C.

Ten days. The lesions are still active but only remnants of the vesicle can be made out about the spreading crust. From this time on the lesions lose their specific characters. The enlargement and induration of the axillary lymph nodes suffer a continuous diminution, although the consistency is greater than normally for some days after the healing of the specific lesion is complete.

Twenty-three days after the first inoculation the animal was inoculated again on the skin of the abdomen with vaccine virus No. 148, but no reaction followed.

No. 101. Adult male *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 246. Body temperature 39° 2 C.

Twenty-four hours after inoculation the scratches show a slight opacity but no elevation. Axillary lymph nodes normal. Body temperature 39° 8 C.

Forty-eight hours. There is now some elevation as well as opacity along the line of inoculation. Axillary lymph nodes normal. Body temperature 37° 8 C.

Three days. The elevation and opacity is more marked and now extends for a distance of 2 or more millimeters on each side of the scratch. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. The lesions present as irregular pink elevations which are surrounded by a narrow linear crust which occupies the site of the inoculation scratch. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Five days. Lesions show a depressed central crust bordered by an elevated zone which is translucent near the crust. The whole lesion measures 8 millimeters across. Axillary lymph nodes enlarged. Body temperature 39° C.

Six days. The central crust has enlarged and the surrounding elevated ring is opaque and vesicular for 2 millimeters about the crust. The areola is bright and the whole lesion sharply circumscribed. Axillary lymph nodes enlarged. Body temperature 39° C.

Seven days. The lesion is the same as yesterday save for an increase in the area of the crust and a filling out of the vesicular ring. Axillary lymph nodes slightly enlarged. Body temperature 40° C.

Eight days. Lesion shows the same features but the whole lesion has ceased to spread. The depression of the central crust is marked. Axillary lymph nodes enlarged and hard. Body temperature 38° 8 C.

Nine days. The crust has been picked off and the lesion presents an excoriated area about which the remnants of the vesicle can be made out. Axillary lymph nodes less enlarged but hard. Body temperature 39° 4 C.

Ten days. The lesions are now healing and have lost their specific characters. Axillary lymph nodes but slightly enlarged. Body temperature 39° 6 C.

The further course of the lesions showed only the healing process. The axillary lymph nodes were of normal size and of only slightly increased consistency. In this case the lymph nodes did not show as much reaction as is usual after skin inoculation with vaccine.

Twenty-three days after the original inoculation the monkey was vaccinated a second time on the skin of the abdomen with vaccine virus No. 148. No reaction followed the second inoculation.

No. 105. Adult male *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with vaccine virus No. 148. Body temperature 40° C.

Twenty-four hours after inoculation the scratches show no reaction. A narrow crust marks the line of each inoculation. Axillary lymph nodes normal. Body temperature 39° 5 C.

Forty-eight hours. There is a slight elevation which is opaque and a faint pink color about the crust. Axillary lymph nodes normal. Body temperature 38° 8 C.

Three days. About the scratch the epithelium is elevated, opaque, and pink for a distance of 2 or more millimeters. On pressure a small amount of clear serum oozes from under the crust. Axillary lymph nodes slightly enlarged. Body temperature 38° C.

Four days. The lesion presents as a sharply circumscribed, pink elevation 3 millimeters in width which surrounds a slightly depressed crust. Axillary lymph nodes enlarged. Body temperature 39° 8 C.

Five days. The central crust has enlarged and there is a translucent vesicular ring. Axillary lymph nodes enlarged and hard. Body temperature 39° 8 C.

Six days. The depressed yellow central crust is now bordered by an elevated ring in which can be distinguished an opaque, yellowish zone which merges with a translucent band of a gray color. The areola is distinct and bright red. Axillary lymph nodes enlarged. Body temperature 39° 6 C.

Seven days. The lesion has attained a total width of 1 centimeter. Some subcutaneous edema is apparent. Axillary lymph nodes enlarged and hard. Body temperature 39° 8 C.

Eight days. Lesions show same features as yesterday. Axillary lymph nodes slightly enlarged. Body temperature 40° 1 C.

Nine days. The crusts have been picked from the lesions and have left an excoriated area surrounded by a ragged, opaque, epithelial edge about which the skin is reddened. Axillary lymph nodes enlarged. Body temperature 39° C.

Ten days. From this time on the lesions show simple repair. The axillary lymph nodes were more or less enlarged and hard for some time.

The monkey was inoculated on the skin of the abdomen with vaccine virus No. 148 on the twenty-third day after the first inoculation. This second vaccination was followed by no reaction.

#### SUMMARY.

(1) *Objective description of the specific skin lesion based upon the appearances in twenty-eight monkeys.*—After 48 hours the lesion appears as a simple scratch with more or less opacity and elevation, not extending over 2 millimeters from the line of inoculation.

After 72 hours the elevation may become less in extent and take on a pink color, or it may remain as before.

After 96 hours the elevation becomes more marked and more sharply circumscribed, and may begin to show translucence about the crust which forms along the line of inoculation.

After 5 days the zone of translucence about the crust is more evident and contains fluid, which forms a vesicular ring about the crust. The contents of this vesicle is a clear fluid. The vesicular ring is bordered externally by a zone of red, which fades peripherally. The whole lesion—i. e., crust, vesicle, and areola—forms a rounded elevation, which flows into the general skin surface without a sharp line of demarcation.

After 6 days the various parts of the lesion remain the same as on the fifth day, but there is an increase in the total width. The vesicle ring spreads peripherally, and coincident with this the crust encroaches upon the inner side of the vesicle ring. The inner part of the vesicle ring may present an opaque appearance at this stage. The crust may be more or less depressed in such a manner that the vesicular ring forms a rampart about it.

After 7 days the conditions remain the same, there being more or less spreading of the whole lesion, accompanied by an increase in the extent of the central crust. At this stage, the outer edge of the vesicular ring may present an abrupt declivity, so that the whole lesion forms a flat-topped plateau. The skin proper is thickened and hard, and a subcutaneous oedema is apparent beneath the lesion.

After 8 days the lateral extension of the lesion has ceased and the central crust has encroached upon the vesicular ring. The subcutaneous oedema noted after 7 days is now more noticeable, often presenting itself as a broad indurated base.

From this time on the healing of the lesion proceeds rapidly. The central crust finally occupies all the space previously held by the vesicular ring, being bordered by the pink epithelium which is growing inward from the normal skin. This newly formed epithelium slowly spreads beneath the crust, and finally the latter falls off, leaving a pink and shining scar.

The lateral excursions of the process outward from the line of inoculation amount to from 6 to 8 millimeters, measuring from the center of the crust to the outer edge of the vesicular ring. After 5 days, when the



lesion is first macroscopically analyzable into zones which correspond with the microscopic findings, the growth of the lesion is seen to be due to a lateral extension of 3 elements. These may be described, from without inward, as—

- (1) A zone of hyperæmia.
- (2) A vesicular ring, composed of—
  - (a) A zone of translucence.
  - (b) A zone of opacity.
- (3) A crust.

Up to the end of the eighth day of the development of the lesion these move out from the line of inoculation in the order named, each encroaching upon its outer neighbor toward the end of this period, a little more rapidly than the latter spreads. On the eighth day of the development of the lesion this spreading stops, and the zone of hyperæmia slowly fades, the opaque zone spreads over the remnant of the translucent zone, and it in turn is taken up by the spreading of the crust.

The profile of the lesion presents 3 rather distinct phases, as follows: After 5 days it is more or less hemispherical, or at least an arc of a circle. After about 7 days it is flat-topped, presenting a steep declivity on either side, with perhaps a rampart effect from the depression of the crust, and after 8 days the whole lesion may be raised upon a broad swelling, which elevates with it some of the normal epithelium about it.

(2) *Histology of the vaccine lesion of the skin.*—The histogenesis of the vaccine lesion in the skin of the monkey is similar to that of the vaccine lesion in the skin of other mammals. We find the same changes in the epithelial cells leading to vesicle formation, followed by reparative processes resulting in complete healing of the lesion. The histology of the vaccine lesion has been described so often that it seems unnecessary to repeat it here. We find the development of the lesion to be the same after inoculation with different strains of virus.

In the study of our large series of vaccinations of the monkey we have been struck by the extent of the process in the corium and in the subcutaneous tissue beneath the lesion. We can recognize a general inflammatory and reparative process, and besides a series of phenomena which we regard as due to the activities of the specific organism. The former group of phenomena are shown by cellular and fluid exudative processes and by proliferation of connective tissue cells with the new formation of blood vessels, and are such as would accompany any infected wound of the skin. Besides these we find early in the process a marked swelling and proliferation of the endothelial cells of the capillaries adjacent to the specific process in the epidermis. The endothelial cells of the capillaries and lymphatics in these situations are frequently invaded by the protoplasmic phases of *cytocyctes variolæ*. This reaction in the corium is in evidence from the third day of the disease onward. We interpret this condition as due both to the action of the toxins set free from the specific

lesion in the epidermis and to an invasion of the blood vessels by the organism.

The process in the corium and in the subcutaneous tissue beneath the lesion does not present such definite areas of necrosis as will be described in the variolous lesions. This may be due to the fact that the vaccine lymph was glycerinated, and so was free from pyogenic organisms, while the variola virus was untreated and contained streptococci and other pathogenic bacteria. It is to be noted that beneath the vaccine lesions capillaries invaded by cytocytes were only found near to the infected epidermis while, as will be shown, in variolation the specific process extends deeply into the subcutaneous tissue.

(3) *Constitutional reaction*.—The general condition of the animal does not seem much disturbed. There may be some anorexia about the sixth day, but it can not be said to be constant or marked.

(4) *The temperature reaction* is not very definite, although there is to be derived from it evidence of a constitutional reaction. The most common reaction is a slight rise on the sixth and ninth days of the disease. This fever rarely exceeds 40° C. The temperature reaction is not nearly so typical as in *variola inoculata*.

(5) *The lymph nodes*.—With the development of a vaccine lesion on the belly of a monkey there is a reaction of the axillary lymph nodes. This is shown by an enlargement, first noticeable on the fourth day (after 3 day's development of the lesion), which becomes more apparent on the succeeding days. On the sixth or seventh day the nodes may be 1 centimeter in diameter and tender. With the regression of the lesion the nodes decrease in size but remain more firm than they are normally.

The histology of the axillary lymph nodes of monkeys vaccinated on the abdomen presents the same picture as that seen in the variolated monkeys. Oedema of the sinuses and the presence of phagocytic endothelial cells, red blood corpuscles, and leucocytes, together with a small amount of fibrin, characterize the process in the nodes taken from animals during the active evolution of the vaccine lesion.

(6) A general exanthem has never occurred in our vaccine monkeys (total 28). In some cases secondary lesions occur about the primary lesion (daughter pocks). These run the same course as the primary lesions. Auto-inoculations are not uncommon, but in all instances which we have observed their occurrence was so obviously due to infection from the initial lesion by acts of the animal that the question of their being of the nature of an exanthem could not be raised.

(7) No lesions were found in the internal organs. The bone marrow and testes were found free from the focal lesions which occur in these organs in *variola vera* in man. *Cytocytes variolæ* was constantly found associated with the specific process in the skin at the site of inoculation. The question of the occurrence of the organism will be considered more in detail in a separate section of this report.

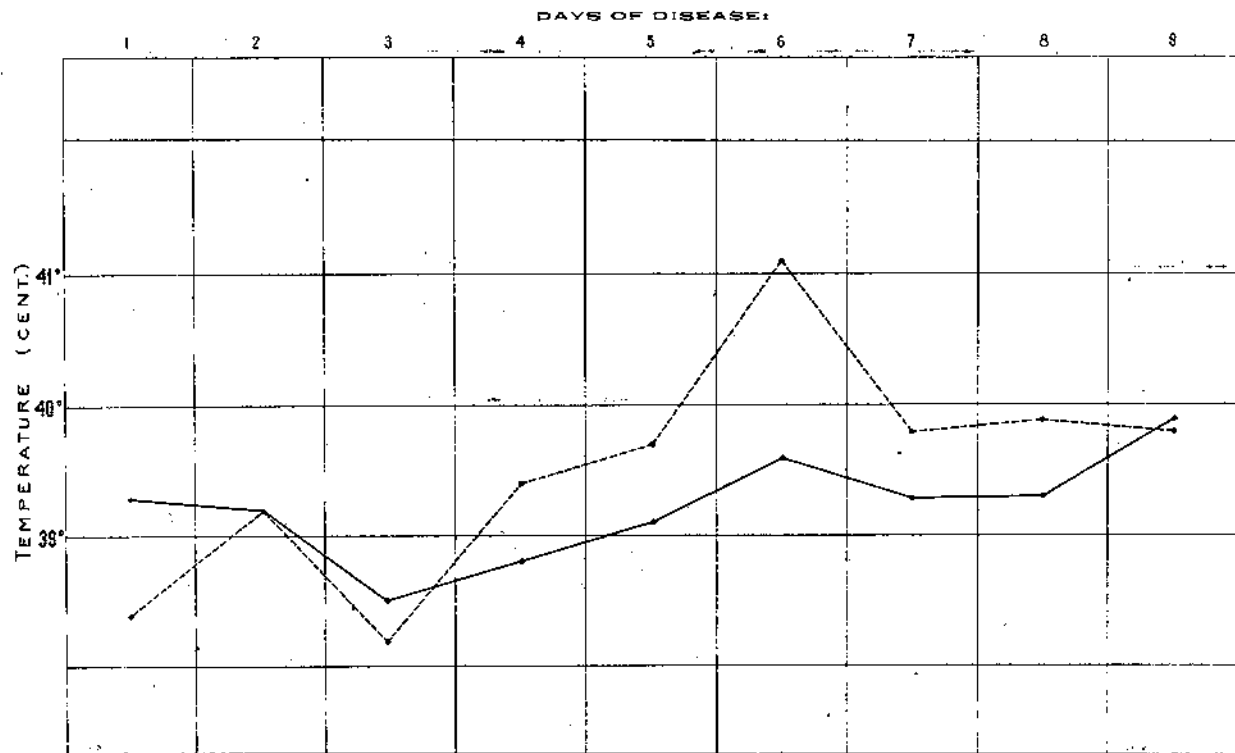
## DISCUSSION.

In considering the results of the preceding experiments certain points of considerable theoretical interest present themselves for discussion. Perhaps the most prominent feature of the disease vaccinia is the specific immunity which it produces. At it is hardly possible to consider this immunity without at the same time dealing with variola, we have decided to discuss this phase of the disease in a separate section. There remains for consideration here the clinical feature of the disease vaccinia as it occurs in *Macacus cynomologus*. In our experiments we have found that this species of monkey reacts to an inoculation with vaccine virus on the skin by a definite sequence of phenomena which are reproduced in each experiment with only very slight modifications. It is true that certain animals do not react in exactly the same degree as do others, but these "abortive" reactions seem to us to depend upon conditions of natural immunity and to differences in the virus used for inoculation, and so do not affect the truth of the general statement that this species is markedly susceptible to the disease.

Comparing vaccinia in *M. cynomologus* with vaccinia in man and in the calf we see that we have a strict parallel. We find a lesion developing at the site of inoculation which runs a definite course and heals after a fairly constant interval. This local lesion is associated with enlargement of the lymph nodes. Only those nodes are affected which are interposed between the areas of the skin on which the lesion develops and the main lymph trunks. During the active evolution of the local lesion there is a general reaction of the inoculated animal as shown by a rise in the body temperature. The primary lesion is shown to exist at the site of inoculation from a very early period after the inoculation as a distinct process so that the latent period, before the lesion can be diagnosed by its appearance to the naked eye, is shown to be rather apparent than real.

Two things serve to differentiate the vaccine process from that following inoculation of variola virus on this species of monkey: First, in vaccinia we have never observed an exanthem; second, the temperature reaction is not so definite in its onset as in *variola inoculata*.

FIG. 1. TEMPERATURE REACTION.



VACCINIA AND VARIOLA INOCULATA IN THE MONKEY. (COMPOSITE CURVES.)

Vaccinia ————— Variola inoculata - - - - -

The histological peculiarities of the specific lesion of vaccinia in these monkeys are similar in every way to those found in lesions following vaccination of other susceptible animals.

We have seen then that the disease vaccinia in *M. cynomologus* conforms strictly to the type of the disease vaccinia and in a more general way can be included in that group of processes which embraces all forms of variola and vaccinia.

The data furnished by our experiments upon the immunity reactions and upon the differences due to different strains of virus will be presented in another section of this article.

#### CONCLUSIONS.

(1) Inoculation of the skin of *M. cynomologus* with vaccine virus is followed by the development of a lesion at the site of inoculation which is similar in all respects to that which follows similar inoculations of other animals.

(2) The development of the lesion is associated with a rise in the body temperature which is most marked during its active evolution.

(3) The lymph nodes which are interposed between the area of skin on which the lesion develops and the main lymph trunks, show enlargement coincidently with the temperature reaction. The nodes show histological changes which account for this enlargement.

(4) *Cytoryctes variolæ* is found in the epithelial cells of the vaccine lesion and also in the endothelial cells of capillaries beneath the epithelium of the lesion.

(5) *Macacus cynomologus* is susceptible to vaccinia.

#### 2. VACCINIAL KERATITIS IN THE MONKEY.

Nine monkeys were inoculated and the animals were killed after various periods to obtain material for the histological study of the lesion at different stages of its development.

*Technique.*—The animal was deeply anæsthetized and the cornea lightly incised with a sharp scalpel. Care was taken not to cut deeply into the corneal substance. A small amount of vaccine was rubbed into the incision. A daily record of the appearances at the site of inoculation and of the general condition of the animal was kept. When the animals were killed a complete autopsy was done, and tissue for histological study was saved from the site of the specific lesion and from the viscera. The animals were under the influence of morphine during the development of the lesion.

#### EXPERIMENTS IN DETAIL.

No. 13. Monkey inoculated on both corneas with vaccine virus No. 1. After 17 hours the cornea shows no macroscopic evidence of a specific process. Animal chloroformed and tissues preserved for histological study.

*Histological examination.*—Sections of the cornea show very slight injury to corneal substance, and the defect completely covered over by epithelium. No evidence of any process other than repair.

No. 10. Monkey inoculated on both corneas with vaccine virus No. 1. Twenty-four hours after the inoculation there is a roughness barely perceptible along the line of incision. Animal chloroformed. When the eyes were dropped into Zenker's fluid, minute opaque spots appear at once along the line of inoculation.

*Histological examination.*—The epithelium has grown over and completely filled the defects in the corneal substance representing the inoculation incisions. Numbers of leucocytes are present in the tissue about the incisions. The epithelium contains many cytoplasmic phases of *cytocytes variolae*, which are rather small and occupy cells which are apparently normal.

No. 11. Monkey inoculated on both corneas with vaccine virus No. 1. Twenty-four hours after inoculation there is slight roughness along the line of incision. After 48 hours there is some elevation as well as roughness and a central loss of substance is also apparent. Animal chloroformed and tissues preserved for histological study.

*Histological examination.*—Section presents two clean cut and moderately deep incisions, one of which is filled with epithelium. The second incision is not filled in and the surface about it is denuded of epithelium for a short distance. Comparatively few polymorphonuclear leucocytes are present about the lesions. The corneal tissue beneath the incisions is oedematous. On one side of the incision, the corneal epithelium is two and a half times thicker than normally. Elsewhere the epithelium is thinner than normal, both near the incisions and for a considerable distance from them. In places, the epithelium is lifted up by an exudate. Cytocytes are present in the cells of the lesion.

No. 7. Monkey inoculated on the right cornea with vaccine virus No. 1. The left cornea inoculated with fresh virus obtained from a vaccine lesion of the skin of Monkey No. 1; duration 4 days.

Twenty-four hours after inoculation the right cornea shows no macroscopic lesion, while the left cornea is slightly opaque and rough along the incision.

Seventy hours. The right cornea shows slight elevation at the site of inoculation. The left cornea is slightly rough along the line of incision. Animal chloroformed. When the eyes were placed in Zenker's fluid a loss of substance was apparent along the lines of inoculation.

*Histological examination.*—The corneal surface is denuded of epithelium over an area 0.5 millimeter across, surrounding the site of the inoculation incision. There is at no point any proliferation or thickening of the epithelial layer. It is thin and tapering at the border of the denuded area, and here cytocytes are present in both the degenerated and the well-preserved epithelial cells. Very few leucocytes are found and when present are usually situated upon the denuded surface of the cornea.

No. 12. Monkey inoculated on both corneas with vaccine virus No. 1.

Forty-eight hours after inoculation there is no appreciable elevation or roughening, but there is distortion of an image reflected on the cornea.

Three days. The left cornea is distinctly rough at the site of the inoculation. The right cornea is very slightly roughened. There is no opacity of the cornea. A small amount of muco-purulent material is present in both conjunctival sacs.

Four days. Condition of corneas the same. Animal killed and tissues preserved for histological study.

*Histological examination.*—Both corneas present areas about 1 to 1.5 millimeters across, from which the epithelium is lost. In the center of this area the corneal connective tissue presents a minute defect representing the trauma of inoculation. The epithelium tapers to a thin edge about the denuded area and here are found cytocytes in small numbers. Leucocytes are practically absent from the lesion.

No. 20. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-four hours after inoculation both corneas are smooth.

Forty-three hours. Slight distortion of reflected image. No conjunctival discharge and no photophobia.

Three days. A slight depression marks the line of inoculation, about which there is some roughening of the corneal epithelium.

Four days. Marked roughening of cornea about inoculation sites with central loss of substance. Photophobia and some muco-purulent discharge noted.

Six days. Condition same, save that the loss of substance is more marked. Animal chloroformed and tissues preserved for histological study.

*Histological examination.*—Sections stained with eosin and methylene blue. Vertical sections taken just outside the edge of the area of the cornea denuded of epithelium show a widespread thickening of the epithelial layer and large numbers of cytocytes at a point nearest the inoculation site.

No. 17. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-three hours after inoculation both corneas are smooth, but the line of inoculation can be made out by the distortion of a reflected image.

Fifty-three hours. Both corneas show slight loss of substance, together with some roughening. A slight degree of photophobia is present.

Three days. The corneas are hazy and the loss of substance at the site of inoculation has increased. There is some photophobia and some muco-purulent discharge.

Four days. Marked roughness of the corneal surface is apparent about the inoculation sites. Photophobia and discharge continue.

Five days. Each cornea shows a shallow erosion, about which the cornea is rough and hazy. Photophobia and discharge still present.

Six days. Condition same as yesterday save that the conjunctivæ are oedematous.

Seven days. Macroscopic appearances as before. Animal chloroformed and tissue saved for histological study.

*Histological examination.*—The condition found is similar to that described in the other corneas of this series. There is an area about the point of inoculation over which the epithelium is wholly deficient. The surface is smooth and the corresponding portion of the cornea is slightly thinner than normal.

The epithelium tapers at the border of the deficiency and at this point contains small numbers of cytocytes. In one cornea there is a deep incision partially filled with epithelium. The tissue about this contains a small number of leucocytes.

No. 10. Monkey inoculated on both corneas with vaccine virus No. 1.

Twenty-four hours after inoculation the cornea appears smooth.

Forty-eight hours. Corneas show slight distortion of the reflected image.

Three days. Both corneas slightly roughened along the line of inoculation.

Four days. Roughness is more marked and is present over a circumscribed area about the incisions. The cornea appears hazy and a small amount of muco-purulent material is present in the conjunctival sac. Slight degree of photophobia is observed.

Five days. Condition same as yesterday, save that no photophobia is noted.

Six days. A loss of substance is evident at the center of the roughened area. The conjunctival discharge and the photophobia persist.

Seven days. The superficial loss of corneal substance has increased, otherwise the condition remains the same.

Eight days. Animal chloroformed. There is considerable œdema of the lids with a watery discharge. The eroded area has increased in extent. Tissues preserved for histological study.

*Histological examination.*—The epithelium covering the central portion of the corneal surface is very thin and consists of only two layers of cells. There is deficiency of epithelium over an area not over 0.5 millimeters across. Cytoryctes are present in considerable numbers in a single area of the epithelium.

#### SUMMARY.

The inoculation of the monkey's cornea with vaccine virus, provided a secondary pyogenic infection does not supervene, results in the production of a lesion which has rather indefinite macroscopic characters. Twenty-four hours after the inoculation a slight roughening, associated with more or less elevation of the corneal epithelium, may be apparent. After 48 hours a loss of epithelium about the point of inoculation is frequently present. The further development of the lesion is associated with an increase in this erosion and with the development of some degree of photophobia and a conjunctival discharge.

The histological characters of the lesion are as follows:

In the corneas taken 17 and 24 hours after vaccination the defect produced by the inoculation trauma is filled in by the growth of the epithelium. This constitutes the ordinary form of repair following simple incision of the cornea. Cytoplasmic forms of Cytoryctes are present in the epithelium. Subsequent to this, up to 8 days and probably for an indefinite period, there is a loss of epithelium over an area surrounding the site of the inoculation. The epithelium is thin and tapers at the edge of the abraded area, and here the cells appear to be undergoing degeneration. Cytoryctes are present in all lesions. Leucocytes apparently play no part in the process but are present in those lesions in which there is injury to corneal connective tissue.

The destruction of epithelium evidently outstrips the growth of the epithelium in the corneal vaccine lesions of the monkey.

#### DISCUSSION.

When we compare the lesion produced on the cornea of the monkey by inoculation with vaccine virus with that which follows a similar inoculation on the rabbit, we see that, although the fundamental characters of the lesion are the same, there are important differences.

One of the striking features of the vaccine process on the monkey's cornea is the loss of epithelium about the line of inoculation. This loss is apparent in the early as well as in the more advanced stages of the process and probably persists until the active stages are passed. It is evident that the vaccine virus in these experiments produced a more extensive destruction of corneal epithelium than has been observed in the



corneal inoculations of the rabbit, in which the growth of the epithelium over compensates any destructive action. The removal of the degenerated epithelium was probably assisted by the dextrous rubbing of the eyes which was frequently observed with the monkeys inoculated upon the cornea.

The course of development of the vaccinal lesion on the monkey's cornea makes it a less favorable place than we had hoped for the study of the etiological factor of the disease. Owing to the loss of epithelium which takes away the bulk of the infected cells the sections are not rich in parasites. In spite of this, the sections from the corneal lesions yielded important data which will be considered in a later article dealing specifically with *Cytoryctes variolæ*.

#### CONCLUSIONS.

(1) Vaccination of the cornea of the monkey produces a lesion which is specific and which is comparable with that following the same inoculation in the rabbit.

(2) The lesion is characterized chiefly by an early loss of epithelium at the site of inoculation, which is accompanied by the development of photophobia and conjunctivitis.

(3) *Cytoryctes vaccinae* are present in the cells of the lesion.

#### 3. VACCINIA FOLLOWING INOCULATION OF THE MUCOUS MEMBRANE OF THE MONKEY.

In this section will be presented the results of inoculation of the mucous membrane of the monkey with vaccine virus. The evolution of the lesion at the site of inoculation, the general reaction of the animal, and the histology of the specific lesion will be described in some detail. These inoculations were undertaken to show what variations might result in the evolution of the specific lesion of vaccinia due to a change in the *locus* of inoculation. The material collected for the histological study of the specific lesion also served for the study of the parasite of the disease.

*Technique.*—Nine monkeys were inoculated in this series. Vaccine virus No. 1 was rubbed into shallow incisions made on the mucous membrane of the soft palate, of the septum of the nose, and of the inner surface of the lower lip. The appearances at the site of inoculation, the body temperature, and the general condition of the animal were noted daily. The monkeys were killed at various intervals after the inoculation, and material for histological study was preserved in Zenker's fluid.

#### EXPERIMENTS IN DETAIL.

No. 81. Adult male *M. cynomolgus*. Inoculated on the left side of nasal septum, on inside of lower lip, and on the soft palate with vaccine virus No. 1. At the time of inoculation some of the virus was also blown into the throat. Body temperature 39°.9 C.

Twenty-four hours after the inoculation no process was visible at the site of the incisions. Body temperature 38°.5 C.

Forty-eight hours. In the nose the inoculation scratch is just visible. The lip presents a small white spot. The palate shows a minute, white papule 1 millimeter or less in diameter. Body temperature 38° C.

Three days. Nose: Some elevation and opacity of the mucous membrane about a small brown area. Lip: Three circular opaque white areas, 2 millimeters in diameter, along the line of inoculation. Each of these opaque areas shows a minute central pink spot. Palate: In the inoculated area are two small, slightly elevated, opaque white spots, about 2 millimeters in diameter, each with a minute central pink dot and surrounded by an areola. Body temperature 38°.4 C.

Four days. Nose: An opaque white elevation, just inside the orifice. Lip: An elevated opaque white area, 3 × 6 millimeters, the center of which is macerated and which is surrounded by a distinct red flush. Palate: An oval area, 3 × 4 millimeters, opaque-white, with 2 minute translucent dots near the center. The 2 areas noted yesterday have fused. Marked peripheral pink flush. Body temperature 30° C.

Five days. Nose: Left orifice almost closed by swelling of mucous membrane. On the anterior portion of the left side of the septum is an elevated, white area surmounted by a small vesicle surrounding a small yellow crust. Lip: The lesion presents an eroded area with an elevated ragged edge, 10 × 5 millimeters in extent and surrounded by a distinct areola. No trace of vesicle formation can be made out, the lesion being essentially an ulcer. Palate: An elevated opaque white area, 5 × 3 millimeters, with gray, semitranslucent center and surrounded by an areola. Body temperature 39°.2 C. Animal chloroformed and autopsy done at once. Primary lesions as described. No evidence of a septic lesion found in gastro-intestinal tract. Viscera normal.

No. 82. Adult male *M. cynomologus*. Inoculated on mucous membrane of nose, lip, and palate with vaccine virus No. 1. Body temperature 39°.5 C.

Twenty-four hours after the inoculation the mucous membrane shows no macroscopic lesion. Body temperature 39°.5 C.

Forty-eight hours. A small, white area is visible in the nose and a similar spot is seen at the site of inoculation on the lip. The palate shows no lesion. Body temperature 38°.8 C.

Three days. Nose: A pink elevation is visible. Lip: A white, opaque area, 2 × 5 millimeters, is apparent along the line of inoculation. The mucous membrane over this area is more or less macerated, and about the area there is a distinct red flush. Palate: A single translucent papular elevation, 2 millimeters in diameter, with a minute opaque central dot is seen on the line of inoculation. Body temperature 39°.2 C.

Four days. Nose: A number of minute elevated opaque areas surrounded by a pink flush. Lip: An excoriated, bleeding area, 6 × 3 millimeters, with an elevated, opaque, white edge and surrounded by a distinct areola. Palate: An opaque, white area with an elevated margin and surrounded by a red areola. Body temperature 39°.5 C.

Five days. Nose: A grayish elevation with a pink margin. Lip: An elevated opaque white area, 10 × 6 millimeters, with some erosion of the surface and with a sharp border which is surrounded by a red areola. Palate: An elevated, gray-yellow area, 8 × 3 millimeters, presenting several minute, bleeding points and a bright red areola.

Six days. Nose: Inspection difficult and findings indefinite. Lip: Lesion as before but somewhat increased in extent. Palate: Lesion as yesterday save that it has spread a little. Body temperature 40° C.

Seven days. Nose: Mucous membrane swollen and presents an opaque, white elevation on the septum. Lip: Lesion is 1 centimeter in diameter, border slightly irregular and overhanging in places, central portion depressed and eroded, showing in places red granulation tissue and in places islands of macerated epithelium. The mucous membrane about the lesion is hyperæmic. Palate: An opaque, gray area,  $10 \times 4$  millimeters, edge elevated and surrounded by an areola. Body temperature  $39^{\circ}.5$  C.

Eight days. Nose: Yellow crusts are visible, but swelling of the mucous membrane renders close inspection impossible. Lip: Lesion is the same as yesterday save that the areola has faded. Palate: Same as before save that areola is absent and the lesion has not extended. Body temperature  $39^{\circ}$  C. Animal chloroformed and autopsy done at once. Lesions at site of inoculation as described above; internal organs show no evidence of specific lesions.

No. 83. Adult male *M. cynomologus*. Inoculated on the septum of the nose, inside of the lip and on the soft palate with vaccine virus No. 1. Body temperature  $39^{\circ}.8$  C.

Twenty-four hours after the inoculation no specific lesion is macroscopically evident. Body temperature  $39^{\circ}.6$  C.

Forty-eight hours. Nose: A narrow red crust is evident. Lip: Palate shows no reaction. Body temperature  $38^{\circ}.8$  C. Animal chloroformed and autopsy done at once. Extensive lesions of tuberculosis were present. This case will be described from the point of view of the intercurrent disease in another article.

No. 84. Adult male *M. cynomologus*. Inoculated in the nose, on the lip and palate with vaccine virus No. 1. Body temperature  $39^{\circ}.5$  C.

Twenty-four hours after the inoculation no evidence of reaction is visible. Body temperature  $39^{\circ}.2$  C.

Forty-eight hours. Nose: Negative. Lip: Small, red, papular elevation on line of inoculation. Palate: Red line marks site of scratch. Body temperature  $38^{\circ}$  C.

Three days. Nose: A small, gray crust is visible on the septum. Lip: 2 opaque, white elevations, 2 millimeters in diameter, are present, one at either end of the line of inoculation. Between these white spots runs a narrow white line. There is considerable reddening of the mucous membrane about the lesion. Palate: 2 small, opaque, white elevations, 1.5 millimeters in diameter, are joined by a narrow red line. Body temperature  $39^{\circ}$  C. Animal chloroformed and an autopsy done at once. Lesions at the site of inoculation as described above. The mucous membrane of the respiratory and digestive tracts examined and no evidence of a vaccine lesion found.

No. 85. Adult male *M. cynomologus*. Inoculated on the inner surface of the lip, on the nasal septum, and on the soft palate with vaccine virus No. 1. Body temperature  $38^{\circ}.8$  C.

Twenty-four hours after the inoculation there is slight redness about the incision on the lip. The nose and the palate are negative. Body temperature  $39^{\circ}$  C.

Forty-eight hours. Nose: A brown crust is visible. Lip: The redness persists. Palate: 2 small yellow papular elevations are seen on the line of the inoculation. Body temperature  $39^{\circ}$  C.

Three days. Nose: Crust is still present. Lip: A small, opaque, white area,  $1 \times 2$  millimeters, seated upon reddened mucous membrane. Palate: 2 opaque, white papular elevations, 2 millimeters in diameter, and elevated 1 millimeter above the general surface, surrounded by a pink flush. Body temperature  $39^{\circ}.2$  C.

Four days. Nose: Small vesicle with a pink, peripheral flush. Lip: Opaque,

white area, 2 × 2 millimeters, with a distinct pink flush about it. Palate: 2 white elevations, 2 millimeters in diameter, flat-topped, and 1 millimeter in height, surrounded by an areola. Body temperature 39°.8 C.

Five days. Nose: Some degree of erosion of the mucous membrane of the septum at the site of inoculation. Lip: An area, 8 × 4 millimeters, presents an elevated, somewhat ragged, white edge, an eroded central portion, and a circumferential, pink flush. Palate: Elevated area, 8 × 3 millimeters, with a grayish-white center and a pink periphery. The nostril presents a vesicle which has spread from the mucous surface out upon the skin. Body temperature 39°.6 C.

Six days. Nose: Left nostril almost occluded by swelling of the mucous membrane and by yellow crusts. Lip: Lesion presents same features as yesterday. Palate: Lesion has enlarged somewhat and the central portion has a yellow color. Body temperature 39°.8 C.

Seven days. Body temperature 39°.5 C. Animal chloroformed. Nose: Along edge of nostril is a shallow excoriation, from which oozes a partly clear and partly cloudy fluid. The septum presents irregular, opaque, white elevations along the line of the inoculation. Lip: Lesion as before, save that loss of substance is more marked and there is some bleeding. Palate: Lesion as before. Viscera appear normal.

No. 86. Full grown female *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° C.

Twenty-four hours after the inoculation no evidence of a specific process is visible on inspection. Body temperature 39°.2 C.

Forty-eight hours. Nose: A narrow crust marks the site of the inoculation. Lip and palate negative. Body temperature 39° C.

Three days. Lesions show no change since yesterday. Body temperature 38° C.

Four days. Nose: White elevation at site of inoculation. Lip and palate negative. Body temperature 39° C.

Five days. Nose: An elevation 2 millimeters wide on septum near edge, with central loss of substance. Lip and palate negative. Body temperature 38°.2 C.

Six days. Nose: Left nostril almost closed by swelling of mucous membrane and yellow crusts. Inspection of interior not possible. Lip and palate negative. Body temperature 39° C.

Seven days. Nose: Edge of nostril presents a shallow erosion partly covered by a yellow crust. Lip and palate negative. Body temperature 39° C.

Eight days. Nose: Excoriation about nostril persists. Swelling of mucous membrane less marked. Lip and palate show no lesion. Body temperature 39° C.

Nine days. Nose: Lesion at edge of nostril from this time on heals like any vaccination of the skin. Animal allowed to survive. Twenty-two days after the first inoculation the monkey was inoculated again with vaccine virus No. 1. No lesion followed.

No. 87. Full grown male *M. cynomologus*. Animal inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° C.

Twenty-four hours after inoculation the nose is negative, while the lip and palate show a red line on the mucosa at the site of the incision. Body temperature 39°.4 C.

Forty-eight hours. Nose: The inoculation scratch is now visible. Lip negative. Palate: Small red area at site of inoculation. Body temperature 38° C.

Three days. Nose: Mucosa pink, lesion not definite. Lip: A white line 3 millimeters long and 1 millimeter broad marks the site of the inoculation. Palate: Shows a white papular elevation 1 millimeter in diameter surrounded by a pink flush. Body temperature 39° C.

Four days. Nose: White elevation with a pink flush about it. Lip negative. Palate: Opaque, white, oval, elevated area,  $2 \times 3$  millimeters, with a translucent center and a pink peripheral flush. Body temperature  $39^{\circ}.2$  C.

Five days. Nose: Left side of the septum and the roof of the left nostril swollen; considerable mucous discharge. A translucent vesicle is present at the edge of the nostril. Lip: No lesion. Palate: A gray-white elevation,  $2 \times 3$  millimeters, surrounded by a pink flush. Body temperature  $40^{\circ}$  C.

Six days. Nose: Left nostril all but occluded by a mass of yellow crusts springing from the septum and the upper border of the nostril. Lip negative. Palate: Oval area with an elevated, opaque, white edge, an eroded, pink center, and a distinct red areola. Body temperature  $40^{\circ}$  C.

Seven days. Nose: Lesions at edge of nostril extending and involving skin. Inspection of mucous membrane not possible on account of crusts and swelling. Lip negative: Palate: Lesion measures  $6 \times 4$  millimeters, edge sharply circumscribed, elevated and gray-white, central portion gray and eroded. Body temperature  $39^{\circ}.5$  C.

Eight days. Nose: Left nostril presents an opaque, white elevation, which bulges out from the septum and from the upper border of the orifice. The lesion at the edge of the nostril has extended somewhat on the skin surface. Lip negative. Palate: Lesion presents same features as yesterday. Body temperature  $39^{\circ}$  C.

Nine days. Nose: Lesion less extensive than before. Lip negative. Palate: Lesion presents same features as yesterday. Body temperature  $39^{\circ}$  C. Animal chloroformed. At autopsy no specific lesions found other than those at sites of inoculation.

No. 88. Adult male *M. cynomologus*. Inoculated inside of nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature  $39^{\circ}$  C.

Twenty-four hours after inoculation no reaction is visible. Body temperature  $38^{\circ}.6$  C.

Forty-eight hours. Nose: A narrow, dark-red crust marks the line of inoculation. Lip: Mucous membrane reddened about scratch. Palate: An area 2 millimeters in diameter with an opaque white center and a deep red flush about it seen at the point of inoculation. Body temperature  $38^{\circ}$  C.

Three days. Nose: Narrow, brownish, depressed line bordered by an elevation the color of the mucous membrane. Lip: A line 1 millimeter wide, opaque-white in color, occupies the site of the scratch. About this line the mucous membrane is reddened. Palate: A narrow, irregular, white line bordered by an areola, the whole somewhat elevated, is visible at the site of inoculation. Body temperature  $38^{\circ}.2$  C.

Four days. Nose: Mucous membrane injected but no definite lesion can be made out. Lip: An opaque, white area,  $6 \times 2$  millimeters with small area of loss of substance and surrounded by a red areola. Palate: An oval area,  $2 \times 4$  millimeters, slightly elevated, and seated on reddened mucous membrane. Body temperature  $38^{\circ}.5$  C.

Five days. Nose: Some muco-purulent discharge from the left nostril. A white elevation with a pink border is visible on the septum. Lip: Opaque, white elevated area,  $8 \times 2$  millimeters, with some superficial loss of substance and with a red areola. Palate: 2 lesions present, one  $6 \times 2$  millimeters and one  $2 \times 2$  millimeters. Both lesions are slightly elevated and present a gray center, a white periphery, and a red areola. Body temperature  $38^{\circ}.8$  C.

Six days. Lesions same as yesterday save that they have extended somewhat. Body temperature  $39^{\circ}$  C. Animal chloroformed and an autopsy done at once. Specific lesions as described. No evidence of vaccinal lesions found in respiratory or digestive tract.

No. 89. Adult male *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 39° 5 C.

Twenty-four hours after inoculation the lip shows slight reddening of the mucous membrane about the scratch. The other sites are negative. Body temperature 39° 5 C.

Forty-eight hours. Nose and palate negative. Lip: 2 small, red, papular elevations, 2 millimeters in diameter, are present. About these the mucous membrane is reddened for a distance of 1 centimeter. Body temperature 39° C.

Three days. Nose: A small crust is visible at the site of inoculation. Lip: An irregular, opaque, white area, from 1 to 3 millimeters broad and 8 millimeters long, surrounded by a red flush, is present. Palate: 2 flat-topped, white elevations, 2 millimeters across and 1 millimeter high, seated on a slightly reddened mucosa, mark the site of the inoculation. Body temperature 39° 2 C.

Four days. Nose: Septum swollen and beset with yellow crusts. Lip: An irregular elevation from 2 to 4 millimeters wide and 12 millimeters long, dirty-white in color, and presenting superficial losses of substance. Lesion sharply circumscribed and surrounded by a deep-red areola. Palate: Lesions have increased in size. Body temperature 39° 6 C. Animal chloroformed. At autopsy no specific lesions found other than those at the site of the inoculations.

No. 90. Adult male *M. cynomologus*. Inoculated in nose, on lip, and on soft palate with vaccine virus No. 1. Body temperature 40° C.

Twenty-six hours after the inoculation the animal was chloroformed. No reaction was visible at the site of inoculation. No visceral lesions were found at autopsy.

#### SUMMARY.

(1) *Macroscopic appearance of specific lesions*.—Lip: After 48 hours there appears a small, white or reddish papule, or simply a red area at the site of inoculation.

After 72 hours the mucous membrane is slightly elevated and opaque for a distance of 2 millimeters on each side of the scratch. There is a faint peripheral flush or areola.

After 4 days the opaque area has spread 1 or 2 millimeters, the areola is more marked, and the edge of the lesion is more or less sharply circumscribed. There may be some erosion of the opaque area and it always has a macerated appearance. From this time on the lesion spreads slowly, presents a rather sharp, elevated, white, opaque border, with a peripheral flush, the central portion being more or less eroded. There is no macroscopic evidence of vesicle formation or of crusting.

On the palate the lesion runs an identical course and begins to heal about the ninth day.

In the nose, the macroscopic appearances are unsatisfactory, owing to the swelling of the mucous membrane which prevents close inspection. When the inoculation is at all near the anterior nares, there is a decided tendency for the lesion to spread out over the skin about the nostril, where it takes on the characteristics of a skin inoculation.

(2) *Constitutional reaction*.—No general constitutional reaction was observed. The temperature reaction was not marked, although there was

slight elevation of the temperature on the fifth, sixth, or seventh day of the disease. No general exanthem was observed.

(3) *Histological examination of the specific lesions and of the viscera.*—Of the three foci chosen for inoculation, in two the lip and the palate yield similar microscopic pictures. In the nose two varieties of lesions are found, depending upon whether the lesion develops upon the portion lined by stratified or by columnar epithelium. In the former case the lesion is similar to that on the lip or palate.

In a general way the lesion on the palate which can be taken as a type of that on a stratified mucosa epithelium suggests a lesion on a hairless skin from which the crust and the other superficial parts have been removed. When we examine in detail lesions of various durations we find a close similarity in them to the vaccine lesion on the skin, both in the cell changes and in the histogenesis of the lesion. For forty-eight hours after the inoculation the only change demonstrable in a small cleft in the submucous connective tissue which is filled by blood and fibrin, with an occasional polynuclear leucocyte. A few leucocytes are also to be found in and about the neighboring vessels and in the overlying epithelium which has closed over the inoculation wound. Later lesions show swelling and degeneration of the epithelial cells over a small area, which increases in size from day to day as the lesion spreads. If we study a lesion of 5 days' duration we find at the periphery some swelling of the individual epithelial cells, which gives place, as we approach the center of the lesion, to various degenerations. In this thickened epithelium at the edge of the lesion we find fluid collecting between the cells in such a manner as to form minute chambers similar to those in the periphery of a skin lesion of the same duration. This is the only approach to vesicle formation presented by these lesions. In the center of the lesion the epithelial cells are quite unrecognizable, and the space is filled by fibrin, leucocytes, and cell detritus. There is often a rather sharp line of demarcation between the swollen but comparatively normal epithelium which borders the lesion and the degenerated central portion. Occasionally islands of pathological, but still recognizable, epithelial cells are found in the necrotic area.

The submucous tissue presents a complicated cell picture. The blood vessels are more or less prominent, in part on account of swelling and proliferation of their endothelial cells and in part from the large number of polynuclear leucocytes within them and migrating through their walls. The connective-tissue cells are more or less swollen and are frequently undergoing mitosis. A considerable number of eosinophile leucocytes are frequently present. Polynuclear leucocytes occur in large numbers but do not form such a prominent part of the picture as they do in the skin lesion. Besides these readily identified cells there are many mononuclear cells present which are not so easily distinguished. The majority

of them seem to belong to the lymphoid-plasma cell series, while some are probably of endothelial origin.

In the older lesions evidence of repair is found. The epithelial cells grow in from the epithelium at the periphery of the lesion and upward from the ducts of the glands. There is active, new formation of blood vessels and of connective tissue beneath the lesion.

In the portion of the nose covered by columnar epithelium, the process is somewhat different from that described above. We find large areas denuded of epithelium and a marked reaction of an inflammatory sort in the tissue beneath. The lack of coherence between the columnar epithelial cells in this situation prevents any marked thickening at the edge of the lesion.

The protoplasmic phases of *cytocytes variolæ* were present in the epithelial cells in lesions of all durations and in all situations. Infected endothelial cells were demonstrable in one lesion. They occurred in a capillary beneath a lesion of six days' duration on the stratified epithelium of the nose.

#### DISCUSSION.

The foregoing experiments show that an inoculation upon the nasal, buccal, or oral mucous membrane of the monkey with vaccine virus produces a characteristic, self-limiting lesion. Histological study of these lesions shows them to be similar to those produced on the skin by vaccination. The differences observed in the vaccine lesion on the mucous membrane from that on the skin are readily explained by the physical conditions at the site of inoculation. The vaccine lesion on the mucous membrane shows no crust or vesicle. The absence of these characters is undoubtedly due to the fact that the epithelium at this site of inoculation does not possess a horny layer, and also to the fact that the surfaces are constantly rubbing one against the other and are bathed with fluid. We have seen that small collections of fluid between the swollen and degenerated cells of the lesion may take place in the vaccine lesions of the mucous membrane. This process is the same as that seen in the peripheral portions of a developing vaccine lesion on the skin. However, as soon as a considerable amount of fluid collects the tendency is for it to escape on the surface, and so no large vesicle forms. The reaction in the tissue beneath the lesion is similar in kind to that beneath a vaccine lesion of the skin, but the process is less in degree. If we assume that the reaction in the corium beneath the skin vaccination is due to substances absorbed from the specific process in the epidermis, it is to be expected that the reaction would be less intense beneath a vaccine lesion on a mucous membrane. In the latter situation the greater part of the products of the process in the epithelium must escape on the surface. The mildness of the constitutional reaction and the slight degree of fever which these animals present is doubtless due to the latter cause. The



constant presence of the cytoplasmic phases of *cytocytes variolæ* in the epithelial cells of the lesion and the absence of nuclear phases is consistent with the hypothesis that the latter are peculiar to variola, for as we will show later, the epithelial cells of the mucous membrane present many examples of the nuclear phases of the organism when variola virus is used for inoculation.

#### CONCLUSIONS.

(1) Vaccination of the monkey, *M. cynomologus*, upon the nasal, oral, or buccal mucous membrane gives rise to a true vaccine lesion similar to that which follows vaccination of the skin.

(2) The vaccine lesion on the mucous membrane shows certain differences from that upon the skin, but these differences are explained by the physical conditions at the *locus* of inoculation.

(3) The presence of protoplasmic phases of *cytocytes variolæ* and the absence of nuclear phases of the organism in the vaccine lesion on the mucous membrane is consistent with the hypothesis that the former cycle is associated with the lesions of vaccinia and that the latter do not occur in such lesions, being peculiar to variola.

## PART II.

### STUDIES UPON EXPERIMENTAL VARIOLA IN MONKEYS (*MACACUS CYNOMOLOGUS* AND *M. NEMESTRINUS*) AND IN THE ORANG-UTAN (*SIMIA SATYRUS*).

#### 1. VARIOLA INOCULATA FOLLOWING INOCULATION OF THE SKIN OF THE MONKEY.

In these experiments particular attention was paid to the evolution of the specific lesion at the site of the inoculation, to the general exanthem, and to the constitutional reaction of the animal. Material was collected for the histological study of the primary lesion, of the exanthem, and of the internal organs.

*Technique.*—The different strains of virus used were obtained from cases of smallpox occurring among the European and native population of Manila and of the provinces. So far as circumstances permitted, the virus was kept on ice from the time of collection until used for inoculation.

In some experiments the contents of the pustule or the dry disk was used, but as a rule the contents of the unclouded vesicle was selected for inoculation. Each strain of virus, except one, was inoculated at least once upon the skin of a fresh monkey to test its potency. The different strains used were as follows:

No. 21. Collected April 6, 1904, from a case of severe *variola vera* in a native male, aged about 20 years, on the twelfth day of the disease.

No. 52. Collected May 4 from a case of severe *variola vera* in an American negro in Bilibid Prison, aged about 30 years, on the tenth day of the disease.

No. 167. Collected from a case of severe *variola vera* in a native male, aged about 20 years. The contents of the unclouded vesicle was collected on the eighth day of the disease, August 6, and again on the following day. The contents of the pustules was collected on the fifteenth day and the disks from the palms and soles on the nineteenth day of the disease.

No. 199. Collected on September 18 from a case of severe *variola vera* in an American infant on the ninth day of the disease.

No. 200. Collected on September 17 from a case of severe *variola vera* in a Spanish-Filipino infant on about the ninth day of the disease.

No. 252. Collected October 7 from a case severe *variola vera* in a native infant, at autopsy, on about the tenth day of the disease.

No. 307. Collected on October 23 from a case of severe *variola vera* in an adult native male on about the ninth day of the disease.

No. 325. Collected November 3 from a case of mild *variola vera* in a native boy, aged 8 years, on about the tenth day of the disease.

No. 326. Collected November 6 from a case of severe *variola vera* in a native boy, aged 12 years, on about the eighth day of the disease.

No. 327. Collected on November 9 from a case of severe *variola vera* in a native girl, aged 11 years, on about the ninth day of the disease.

*Method of inoculation.*—The introduction of the contagion into the animal was effected by making a number of separate shallow scratches with the point of a scalpel on the previously shaved and cleaned skin of the abdomen, and rubbing the virus into the wound with the back of the instrument. The inoculations were placed at least 2 centimeters apart. The usual precautions were observed to avoid the introduction of extraneous matter. An anaesthetic was employed whenever discomfort might otherwise be caused to the animal.

The animals were observed daily during the course of each experiment. The appearance of the lesions to the naked eye at the sites of inoculation was recorded, together with the occurrence and evolution of the exanthem, the constitutional reaction, the body temperature taken per rectum, and the reaction of the lymph nodes.

Material for histological study was obtained either by excision of the lesions during the course of the experiment, or by autopsy when the animal was killed. All tissues were fixed in Zenker's fluid for twenty-four hours, washed in running water over night, and then hardened by passing through alcohols of graded strength. Tissues were embedded by the chloroform-paraffine method. Sections were cut on the Minot microtome of a thickness of from 3 to 6 microns, and were stained in a variety of ways.

In certain experiments the immunity of the animal, resulting from the first inoculation, was tested by subsequent skin inoculations with vaccine or variola virus.

This section is based upon the study of sixty-five monkeys inoculated on the skin with variola virus, of which the following experiments are selected to be given in detail.

#### 1. *Clinical course of the disease.*

No. 114. Adult, male, *Macacus cynomologus*. Monkey was inoculated in 12 places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 37°.8 C.

Twenty-four hours after inoculation the scratches show a narrow, dry crust, about which the skin is opaque and slightly elevated for a distance of 1 or 2 millimeters. Body temperature 39°.2 C.

Forty-eight hours. The skin for a short distance about the crusts is white, but this area fades, without a definite line of demarcation, into the surrounding normal skin. Body temperature 38°.2 C.

Three days. The elevated skin about the crust is pink for a distance of 2 millimeters. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. The lesions are present as rounded, pink elevations with fairly definite borders and surmounted by delicate yellow crusts. The lesions average

7 millimeters across. In some there is a narrow translucent zone immediately around the crust. Axillary lymph nodes somewhat enlarged and firm. Body temperature 40° C.

Five days. The central crust is depressed, and is surrounded by a definitely elevated vesicular ring, which is bordered externally by a red areola, fading outwards into normal skin. There is much œdema of the subcutaneous tissue beneath the lesions, producing a broad indurated base on which the lesions are individually prominent. The lesions average 8 millimeters in width. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41° 5 C.

Six days. Both the crust and the vesicle have increased in extent. The contour of the lesion shows an abrupt elevation in the zone of the areola. Average width, 10 millimeters. A few small red papules are noted on the shaved area of the abdomen near the belt line. Axillary lymph nodes enlarged and hard. Body temperature 40° 8 C.

Seven days. The lesion presents as a flat-topped elevation with a central brown crust and an opaque vesicular ring. Average width 8 millimeters. The lesions are less prominent to-day, owing to the subsidence of the subcutaneous œdema. Two of the papules noted near the belt line have increased somewhat in size. Axillary lymph nodes as before. Body temperature 40° C.

Eight days. Primary lesions are beginning to undergo involution, the crust is spreading at the expense of the vesicular ring. Subcutaneous œdema has almost disappeared. On the face there are a dozen or more papules and vesicles from 2 to 4 millimeters in diameter. Similar lesions are present on the abdomen, the scrotum, the inner aspect of the thighs, and on the palms. Axillary lymph nodes as before. Body temperature 40° C.

Nine days. The vesicular element in the primary lesions has been obliterated by the spreading of the central crust. The lesions of the exanthem are present as filled out vesicles, some of which have opaque, white or yellow contents, and all are surrounded by a bright-red areola. An abundant eruption is noted on the tail and the skin about its base. Axillary lymph nodes slightly enlarged and hard. Body temperature 39° 5 C.

Ten days. Many of the primary lesions have been scratched and are present as shallow ulcerations over which the epithelium is spreading from the edge. The lesions no longer present specific characters. The lesions of the exanthem are dry and crusted. The eruption is noted to-day on the soles and on the dorsal aspect of several of the fingers and toes. Axillary lymph nodes of almost normal size but still firmer than normally. Body temperature 38° 5 C.

From this time on the specific lesions healed without complications. Material was collected at intervals for the histological study of the primary lesions.

No. 115. Half-grown male, *Macacus cynomologus*. Inoculated in 12 places on the abdomen with virus No. 167 (vesicle contents). Body temperature 38° C.

Eighteen hours after the inoculation a narrow, brown crust, surrounded by a sharply circumscribed, elevated, opaque, white area 5 millimeters across, marks the site of the inoculation. Body temperature 39° 4 C.

Forty-eight hours. The skin about the crust is elevated and pink for a distance of 2 millimeters. Body temperature 37° 8 C.

Three days. The elevated area has increased in extent and is distinctly red in color. Axillary lymph nodes slightly enlarged. Body temperature 38° 8 C.

Four days. Immediately about the crust there is a distinct translucence of the skin. Average width of lesions, 7 millimeters. Axillary lymph nodes distinctly enlarged. Body temperature 39° 5 C.

Five days. The lesion is present as a rounded elevation surmounted by a narrow, brown crust, about which there is a distinct vesicular ring, translucent

near the crust and shading insensibly into a pink zone, which fades out into the surrounding normal skin. Considerable subcutaneous cedema. Average width of lesions, 8 millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 41°.5 C.

Six days. The central crust is surrounded by a narrow, opaque, white vesicle on a pink elevation, measuring from 9 to 10 millimeters across. Axillary lymph nodes as before. Body temperature 39°.8 C.

Seven days. The lesions present as flat-topped, sharply circumscribed elevations, from 9 to 10 millimeters in diameter. The central crust, somewhat depressed, is surrounded by an elevated vesicular ring which is opaque-white in color. On the upper lip, the abdomen, the arm, and the scrotum are seen small, red, papular elevations. Axillary lymph nodes as before. Body temperature 39°.8 C.

Eight days. Yellow, turbid fluid oozes from beneath the central crust. The vesicular ring has been obliterated in places by the spreading of the crust. The lesions of the exanthem are somewhat larger and vesicular. Three new lesions have appeared on the face and one in the groin. Lymph nodes as before. Body temperature 39°.5 C.

Nine days. The vesicular zone of the primary lesion has been entirely obliterated. The subcutaneous cedema has almost disappeared. To-day an exanthem is noted at the edge of the nostril and in the vestibule. The tail and the skin at its base present numerous small papules and vesicles, each surrounded by a distinct areola. Axillary lymph nodes enlarged and hard. Body temperature 39°.5 C.

Ten days. Primary lesions are healing and present no specific characters. The lesions of the exanthem have dried and are healing. One eruptive lesion found on the sole of the foot and several on the dorsal aspect of the toes. Axillary lymph nodes of almost normal size but still firm. Body temperature 39°.5 C.

No. 116. Young, adult male, *Macacus cynomologus*. Inoculated in 12 places on the skin of the abdomen with virus No. 167 (vesicle contents). Body temperature 38°.5 C.

Fifteen days after the inoculation the skin about the scratch is slightly elevated and opaque for distance of 2 millimeters. Body temperature 38°.6 C.

Forty-eight hours. The site of inoculation is marked by a narrow, yellow crust surrounded by a pink elevation 3 millimeters in width. Body temperature 37°.8 C.

Three days. The primary lesion as before, save that the elevation is more marked and the color deeper. Axillary lymph nodes slightly enlarged. Body temperature 40° C.

Four days. The brown, central crust is seated upon a pink elevation 6 to 10 millimeters across, which fades out into the normal skin. Immediately about the crust there is a narrow zone of translucence suggesting vesicle formation. A marked subcutaneous cedema renders the lesions prominent. Axillary lymph nodes enlarged and hard. Body temperature 40° C.

Five days. Primary lesion as before but it has increased in size. Axillary lymph nodes markedly enlarged and hard. Body temperature 41° C.

Six days. The crust is surrounded by a definite vesicular ring, which in turn is surrounded by a dull pink areola. Average width of lesions, 9 millimeters. Axillary lymph nodes as before. Body temperature 39°.5 C.

Seven days. The primary lesions present as circumscribed elevations with a central, depressed, brown crust, an elevated, opaque-white, vesicular ring, and a bright-red areola. Average width of lesions 9 millimeters. On the face, the abdomen, the scrotum, the thighs, and the arms are numerous small papules or vesicles, each surrounded by a red areola. Axillary lymph nodes as before. Body temperature 39°.8 C.

Eight days. Primary lesions no longer show a vesicle. Many of the papules have become vesicles and the vesicles pustules. There is some increase in size of the lesions of the exanthem. Axillary lymph nodes as before. Body temperature 40° C.

Nine days. Some subcutaneous oedema persists beneath the primary lesions, which, however, have lost their specific character. The exanthem is beginning to dry on the face. Many papules and vesicles, not noted before, are visible on the tail and on the skin at its base. Axillary lymph nodes enlarged and hard. Body temperature 39° 5 C.

Ten days. The primary lesions are healing. Eruptive lesions found to-day on soles and palms. Axillary lymph nodes still enlarged and hard. Body temperature 39° 6 C.

No. 117. Adult male, *Macacus cynomologus*. Inoculated with same virus and in the same way as the preceding animals. Body temperature 37° 8 C.

Eighteen hours after inoculation a narrow yellow crust is seen upon an opaque, slightly elevated area. Body temperature 39° 2 C.

Forty-eight hours. The elevation and opacity have extended somewhat. Body temperature 37° 2 C.

Three days. The lesion presents a linear crust on a slightly elevated, pink area 4 millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. About the crust is a narrow translucent zone which merges with the pink elevation, which in turn fades off into the surrounding skin. Average width of lesions, 7 millimeters. Axillary lymph nodes distinctly enlarged and hard. Body temperature 39° 5 C.

Five days. A distinct but narrow vesicle is evident around the crust. Considerable oedema beneath the lesions. Areola well marked. Axillary lymph nodes as before. Body temperature 40° 5 C.

Six days. Certain of the lesions show a typical development presenting a crust, a definite vesicle, and an areola, the whole lesion being sharply elevated from the surrounding skin. Other lesions show departure from the normal type in that the vesiculation is less marked and the lesions are not as sharply circumscribed. Lymph nodes enlarged and hard. Body temperature 40° 2 C.

Seven days. Some lesions typical, others show a spreading of the crust without macroscopic evidence of vesicle formation. Average width of lesions 9 millimeters. On the face, abdomen, scrotum, inner aspect of thighs, axilla, and arms are numerous pink, papular elevations 2 millimeters in diameter, some of which present translucent central points. Axillary lymph nodes as before. Body temperature 39° 6 C.

Eight days. Certain of the primary lesions show remnants of the vesicle at the edge of the spreading central crust. The lesions of the exanthem have increased in size and the contents of many of the vesicles has become cloudy. New eruptive lesions are present on the abdomen. Axillary lymph nodes as before. Body temperature 39° 8 C.

Nine days. The primary lesions show a narrow zone of translucent epithelium close to the crust. This appearance suggests the beginning of the healing of the lesion by the ingrowth of new epithelium rather than vesicle formation. Lesions of the exanthem have dried up with or without rupturing. Axillary lymph nodes slightly enlarged but firmer than normal. Body temperature 39° 5 C.

Ten days. The healing of the primary lesions is well under way, and they have lost all specific character. To-day an exanthem is noted in the region about the ischial tuberosities and beneath the tail. Eleven eruptive lesions found in the palms and soles and many are also present on the fingers and toes. Axillary

lymph nodes slightly enlarged and hard. Body temperature 39° C. Specific lesions healed without complications.

No. 118. Adult, male, *Macacus cynomologus*. Inoculated with the same virus and in the same manner as the previous animals. Body temperature 38°.8 C.

Eighteen hours after inoculation. Some elevation and opacity is evident about the scratch. Body temperature 38°.5 C.

Forty-eight hours. Elevation and opacity more marked. Body temperature 37°.4 C.

Three days. Lesion presents as a narrow, yellow crust on a pink elevation 5 millimeters across. Axillary lymph nodes slightly enlarged. Body temperature 39° C.

Four days. A narrow zone of translucence is visible near the central crust. The lesion is roundly elevated, of a pink color, and merges without sharp line of demarcation with the surrounding skin. Average width of lesion, 7 millimeters. Considerable subcutaneous oedema. Axillary lymph nodes enlarged. Body temperature 39°.6 C.

Five days. A definite vesicular ring surrounds the crust and is surrounded in turn by a pink areola. The lesion is not sharply circumscribed. Axillary lymph nodes enlarged and hard. Body temperature 41°.2 C.

Six days. The vesicular ring has become opaque and has increased in extent. There is some diversity in the size of the lesions. Axillary lymph nodes as before. Body temperature 40°.6 C.

Seven days. The central crust is surrounded by an elevated ring in which evidence of vesiculation can be made out in places. The edge of the lesion is sharply circumscribed and rises abruptly from the surrounding skin. A definite pink areola is present. Average width of lesions, 9 millimeters. On the face, inner aspect of arms, thighs, and abdomen are red, papular elevations 2 millimeters in diameter. Some of these present a minute translucent center. Axillary lymph nodes as before. Body temperature 40°.2 C.

Eight days. Involution of primary lesions has begun. The vesicular ring has entirely been obliterated by the spreading of the central crust. The subcutaneous oedema has disappeared. The lesions of the exanthem have increased in size and are definitely vesicular. Some new papules are present today upon the abdomen and face. Axillary lymph nodes of normal size but firmer than normal. Body temperature 40°.2 C.

Nine days. Primary lesions beginning to heal. The exanthem is dry and no new lesions have appeared. Axillary lymph nodes as before. Body temperature 39° C.

Ten days. Primary lesions not notable. Eruptive lesions, not noted before, are present on the palms. Axillary lymph nodes as before. Body temperature 39° C.

No. 170. Adult male, *Macacus cynomologus*. Inoculated in 6 places on the skin of the abdomen with a suspension of pulverized disks (virus No. 167) in sterile salt solution.

Three days. An opaque elevation 3 millimeters across is present at the side of inoculation. Body temperature 39° C.

Five days. Lesion presents a small crust on a pink elevation 5 millimeters across. A narrow zone of translucence borders the crust. Body temperature 39° C.

Six days. The central crust is depressed and about it there is irregular vesicle formation. The lesions vary much in size. Body temperature 40° C.

Seven days. Primary lesions have increased slightly in extent. Considerable subcutaneous oedema is present. Body temperature 39°.5 C.

Eight days. Some of the primary lesions show a distinct but irregularly developed vesicular zone. Near anus is a single vesicle 2 millimeters across surrounded by a red areola.

Nine days. The central crust has encroached upon and partly obliterated the vesicular zone. Single small vesicles are present on the arm, the thigh, beneath the tail, and on the ankle.

Ten days. Primary lesions healing. No new exanthem.

No. 171. Inoculated with the same virus and in the same manner as the preceding monkey.

The evolution of the primary lesion was delayed and the vesiculation irregular, as in the previous experiment. No general exanthem was observed. The temperature reaction was indefinite.

No. 164. Half grown male, *Macacus cynomologus*. Inoculated in 6 places on the skin of the abdomen with a suspension of pustular contents [(virus No. 167), dried with lycopodium powder] in sterile salt solution.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratches.

Subsequent to this, for a period of 5 days after the inoculation, there is no evidence of a process.

Six days. Lesions present a narrow crust on a pink elevation. The appearance is the same as that seen on the third day after an inoculation with fresh vesicle contents.

Seven days. The lesion has increased somewhat in extent and is sharply circumscribed.

Eight days. An irregular vesicular zone is developed about the crust.

Ten days. Vesiculation no longer apparent. But slight induration can be made out in the lesion. No general exanthem was observed either before or after this date. (The animal was subsequently inoculated with vaccine virus No. 1, but failed to react.

No. 194. Young male, *Macacus nemistrinus*. Inoculated in 12 places on the skin of the abdomen with virus No. 199. Body temperature 38°.8 C.

Twenty-four hours after inoculation there is slight elevation and opacity about the scratch. Body temperature 37°.8 C.

Forty-eight hours. Marked elevation and opacity around the linear crust. Body temperature 39°.1 C.

Three days. There is marked subcutaneous œdema beneath the lesion. The elevation about the crust is distinctly pink in color. Body temperature 38°.9 C.

Four days. The lesion consists of a rounded, pink elevation 5 millimeters across, surmounted by a crust about which is a narrow zone of translucence, the whole lesion being underlaid by a broad indurated area of subcutaneous œdema. Body temperature 39°.3 C.

Five days. An irregular development of the vesicular ring is apparent in certain of the lesions. Body temperature 39°.5 C.

Six days. Many of the primary lesions have been scratched by the animal and are present as excoriated areas surrounded by a red elevation and seated upon a broad indurated base. Body temperature 39°.6 C.

Seven days. Primary lesions as before. A profuse exanthem consisting of small, red papules and vesicles is present upon the face, trunk, and extremities. Body temperature 38°.7 C.

Eight days. The primary lesions have crusted and in places there is slight vesicle formation. Several new papules and vesicles are present on the face and thighs. One small vesicle is visible on the hard palate. Body temperature 38° C.



Animal killed and autopsy done at once. Material saved for histological examination of specific lesions and of the viscera. Axillary lymph nodes enlarged and red. On section much blood-stained fluid exudes from the cut surface.

## II. *Histological examination.*

The primary lesion 18 hours after inoculation: Microscopic examination of sections from the site of the inoculation shows a solution of continuity which involves the epithelium and may or may not extend into the corium beneath. The defect is filled by an exudate composed of polynuclear leucocytes which lie in a meshwork of fibrin. On the surface the elements of this exudate are fused into a crust. On either side of the defect the epithelial cells of the rete are more or less swollen, and some present clear, circular areas in their protoplasm, suggesting hydropic degeneration. The nuclei of these cells are swollen and the chromatin tends to collect in masses. Polynuclear leucocytes are present in considerable numbers in and around the vessels of the corium and in the connective tissue beneath the defect. The leucocytes are streaming into the defect and into the adjacent epithelium.

Forty-eight hours. The sections present the same general characters as those from the earlier lesions. The defect in the epithelium is sometimes obliterated in such a manner that a layer of epithelium is interposed between the exudate which has collected beneath the crust and the injury to the corium. The polynuclear leucocyte infiltration is more intense. The epithelial cells of the lower layers of the epidermis about the line of inoculation show more marked degeneration, and the cells are frequently separated one from another by fluid.

Three days. The crust has increased in thickness and, with the destruction of the epithelial cells in the central line of the lesion, may fuse with the corium. The epithelial cells of the lower layers adjacent to the crust show various forms of degeneration. Some present ballooning degeneration, and the accumulation of fluid between the cells is more evident than in the earlier lesions. In places, vesicle formation is foreshadowed by the appearance of small, irregular cavities formed either by the accumulation of fluid between the cells or by the bursting of hydropic cells. The corium is the site of a definite reaction, shown by an enlargement of the endothelial cells of the blood and lymph vessels. These enlarged endothelial cells may contain cytotocytes. There is a marked polynuclear leucocyte infiltration of the corium and the epithelium about the crust. In some cases a mass of leucocytes, with granular precipitate and fibrin, is found in the corium just beneath the center of the lesion. The cellular sheaths of the hair follicles show changes similar to those seen in the cells about the crust, and an abundant polymorphonuclear infiltration is present. Some lesions of this age show definite vesicle formation such as will be described later.

Four days. Lesions of this age may present vesicular cavities under the lateral expansions of the crust. In some lesions the whole crust is elevated and the lateral vesicles communicate with one another. The epithelium at the sides of the lesion and below the lateral portions of the vesicle is more or less swollen and shows various degenerations, the most marked forms of which are found in those cells nearest the center of the lesion. The epithelium is completely destroyed in the axis of the lesion. The corium beneath shows proliferation of the endothelial cells of the blood vessels and of the lymphatics, together with an enlargement of the connective-tissue cells. A polymorphonuclear leucocyte infiltration is apparent in the corium and in the thickened epithelium about the vesicle. In some lesions the contents of the vesicle shows a predominance of polymorphonuclear leucocytes under the crust, while the outer portions are all but free from these cells, containing only granular precipitate and fibrin.

Five days. The topography of the lesion at this stage shows considerable variation, due to differences in the extent of the vesicle formation. The typical

picture is similar to that in the four-day lesion with some increase in the extent of the vesicle and in a more marked reaction in the corium beneath the lesion. The polynuclear leucocyte infiltration of the corium is less intense, but the vesicle cavity shows a more even distribution of the pus cells. The reaction of the corium is more marked, being shown by the presence of oedema, necrosis, and an increase in the cellular content of the tissue. Aside from the polynuclear leucocyte the dominant element is a large cell with a vesicular nucleus surrounded by an abundant reticular protoplasm. These cells show a great variation in form, some being rounded, others irregular, the outline seemingly being conditioned by the space in which the cell lies. Many of these cells are found just outside of the capillaries and in the lymph spaces of the tissue, and by comparison of their morphology and staining reaction with that of the swollen endothelial cells, *in situ* in the capillaries and in the lymph spaces, it seems certain that they are identical with them. Many of these cells are phagocytic and in some mitosis is seen. In certain of these cells early stages of the cytoplasmic phases of *Cytoryctes variolæ* are present. In addition to these endothelial cells, which have often wandered for some distance from their place of origin, a certain number of elements are found of the lymphoid and plasma cell series. The connective-tissue cells all through the corium about the lesion are swollen, and in some mitosis is in progress. The nerve bundles are frequently invaded by polymorphonuclear leucocytes. The cellular reaction of the corium is shared by the adjacent subcutaneous tissue and extends for a considerable distance from the line of inoculation. Necrosis is seen in the tissue beneath the center of the lesion, the cells losing their basic affinity and undergoing more or less fragmentation or solution. Many deeply stained spherules of various sizes are scattered through the necrotic tissue, evidently the nuclear fragments of polynuclear leucocytes.

Six days. The general relations of the vesicle cavities and the crust remain as before, although the outer limits of the vesicle often extend beyond the limits of the crust, being roofed by a layer of cornified epithelium which sweeps downward to form a part of the lateral wall of the cavity. In some instances the beginning of vesicle formation, shown by collection of fluid between the cells, is apparent a short distance beyond the outer limits of the main vesicle. A similar condition is met with in the depths of the cellular sheaths of the hair follicles. The fusion of the middle portion of the crust with the underlying corium is often apparent, although in many places a collection of leucocytes is present here which is continuous with the lateral vesicles and with the purulent focus which forms in the corium along the line of inoculation. In such a lesion a roughly T-shaped cavity can be made out, the vertical portion being filled with leucocytes and extending from the crust for a variable distance into the corium, while the cross arm has for its extremities the vesicular cavities which extend laterally under the crust. The vertical portion of this T can often be traced to the focus of necrosis which lies in the lower layers of the corium and the upper portion of the subcutaneous tissue. The reaction of the corium is very marked at this stage. Study of the outer limits of the necrotic area shows that the large cells, which we believe to be endothelial in origin, seem to be resistant to the agent which is causing the necrosis. The large cells are frequently to be seen in an apparently normal condition in an area where all other cellular elements have been destroyed. It is hard to determine whether this apparent immunity from destruction is due to qualities of the cell or whether it is because the cells have migrated into the necrotic area. In one instance a small capillary was found in the corium near the necrotic area lined by swollen endothelial cells, many of which were infected with early stages of the cytoplasmic phases of *cytoryctes variolæ*.

Seven days. The lesions of this age and those collected later show a progres-

sive healing of the process. The vesicle soon disappears and the polynuclear leucocyte infiltration of the whole field becomes less intense and eosinophiles appear.

The epithelium grows inward under the crust and is joined by that which comes from the proliferation of cells of the hair sheaths. The blood vessels of the corium, send out prolongations, and the usual phenomena of repair dominate the picture.

The exanthem: Sections of lesions collected on the first day of the appearance of the exanthem show a vesicular cavity of variable size in the epidermis. This cavity is roofed by a layer of cornified epithelium and laterally and below is surrounded by thickened epithelium. The cells adjacent to the cavity show varying degrees of degeneration. In every case the layer of the *rete* which forms the floor of the vesicle is wanting at one or more points, so that the cavity in the epidermis is in communication with the corium. The blood vessels and the lymphatics of the corium beneath the lesion present marked changes. These are in part due to the migration of polynuclear leucocytes which is going on from the vessels into the corium, the vesicle, and the epithelium about it. Besides this purely exudative phenomenon the endothelial cells of the capillaries and of the lymphatics show marked swelling and some proliferation. The normal relationships of structure in the corium are much disturbed by this combination of exudation, proliferation, and swelling. A careful search of such areas failed to show any cytocytes, although they were numerous in the cells of the *rete* which formed the floor of the vesicle.

Sections from the exanthem collected later in the evolution of the lesion show an increase in the size of the vesicular cavity, but no characters other than those found in the early lesions.

The study of sections from the primary lesions and of the exanthem in *M. nemestrinus* show variations in degree but not in kind from the picture seen in the corresponding lesions in the Philippine monkey. The reaction in the subcutaneous tissue and in the corium is more marked and oedema plays a more important rôle. The vesicle is not so well developed, but is similar in all fundamental characteristics to that in *M. cynomologus*.

Axillary lymph nodes: The sinuses are dilated, sometimes to a high degree. The cell content of the sinuses shows various deviations from the normal. The most prominent character is an increase in the number of endothelial cells. These cells increase greatly in size, become free in the sinuses, and show marked phagocytic properties. In the nodes from monkeys killed on the sixth and eighth days of the disease the included cells are in part red blood corpuscles and in part polynuclear leucocytes. Later the polynuclear leucocytes are the common inclusion, though lymphoid and other cells may occasionally be found within the phagocytes. Besides the endothelial phagocytic cells, red blood corpuscles, and polymorphonuclear leucocytes are found in considerable numbers free in the sinuses. The latter cells predominate in the nodes from animals killed on the ninth day of the disease and later. Eosinophile cells are frequently encountered, but do not as a rule occur in such numbers as do the polymorphonuclear leucocytes.

The follicles show many phagocytic endothelial cells, singly or in small groups, scattered through their substance. In nodes from animals killed on the eighth day of the disease, small areas of hemorrhage were frequently found. In these areas red blood corpuscles were present in the follicular tissue about a small capillary, many of them having been taken up by phagocytes. In nodes collected later in the disease large phagocytic endothelial cells were demonstrable, whose whole cell body was crowded with red blood corpuscles in various stages of dissolution. In one node, collected on the eighth day of the disease, masses of eosinophile leucocytes were present in the follicles and in the sinuses. These cells differed from the usual eosinophile cells in that the granules were elongated.

Eosinophile cells having round, or slightly oval, granules were also present. The cells with the long granules differed from the usual eosinophile cells in having a more distinct cell membrane than the regular type of eosinophile. In this respect they conformed more closely to the type of the polymorphonuclear leucocyte.

Bone marrow and testicle: As these organs present the only specific visceral lesions of smallpox in man they were scrutinized with care in our variolated monkeys. No macroscopic evidence of focal lesions in these organs were seen at autopsy, and the bone marrow from four cases and the testicle from twelve were negative in this respect when studied microscopically.

Liver, spleen, kidney, and lung: No pathological process was demonstrable either macroscopically or microscopically in these organs.

#### SUMMARY.

1. (a) *The macroscopic appearance of the primary lesion in Macacus cynomologus.*—Twenty-four hours after inoculation there is some elevation and some opacity of the skin about the scratch. The process has rarely a lateral extent of more than 2 millimeters.

After 48 hours the appearance is usually the same.

After 3 days the elevation is more marked and the opacity gives place to a pink or red appearance. So far as can be discovered by the naked eye, the specific process may now be said to have begun. Previous to this the reaction has been in no way different from that which follows a simple scratch, without the introduction of virus.

After 4 days the lesion usually attains a breadth of 6 or 7 millimeters and is present as a distinctly pink or red elevation, which is firm to the touch and bears on its summit a narrow crust which has its origin in the drying of the serum exuding at the time of inoculation. However, the crust has somewhat increased in extent. At this time the skin about the crust is more or less translucent. It is often impossible to say whether or not this translucence is, at this time, the site of a definite vesicle.

After 5 days the lesions show the characteristics of a pock, being resolvable into rather distinct zones which correspond with those distinguishable in the microscopic sections. Going from the center of the lesion toward the periphery, we recognize in turn—first a crust, second a vesicular ring, third a zone of elevation and hyperæmia. The lesion now has a lateral extent of from 6 to 8 millimeters. The whole lesion is more or less elevated upon a broad indurated base, due to œdema of the skin and subcutaneous tissues.

After 6 days the picture is the same, save that each of the zones has extended peripherally. At about this time the profile of the lesion undergoes a change in that it loses its flowing outline as a more or less hemispherical elevation, and takes on a flat-topped, plateau-like appearance. In going from the center outward we pass along the fairly flat crust, then over the vesicular ring, which attains a greater elevation than the crust, forming a rampart, and then come to an abrupt declivity where the zone of hyperæmia or areola extends into normal skin.

After 7 days the crust and the vesicular ring will be seen to have extended somewhat, but the former has encroached more or less upon the area occupied by the latter. The subcutaneous cedema is less marked.

After 8 days the lateral excursion of the lesion has definitely ceased and involution begins. This process of involution is evidenced by the spreading of the crust so that it finally occupies all the territory held by the vesicular ring. At the same time the zone of hyperæmia fades the subcutaneous cedema disappears, and the lesions eventually consist of a crust of variable thickness, beneath which the normal epithelium is slowly spreading. If a few days later the crust be forcibly removed, either a small pocket of pus or a tough mass, adherent to the crust, will be found beneath, bordered by pink, new-formed epithelium which is growing in from the periphery. In some lesions the microscopic characters of the process are reflected in the appearances as presented to the naked eye to a greater extent than in others. Thus, in the vesicular ring two zones can at times be made out, an inner, opaque one and an outer, translucent zone. This agrees with the microscopic findings in some lesions where the leucocytes are seen beneath and to one side of the crust, while there is only clear serum in the peripheral part of the vesicle.

Departures from the type lesion were seen in certain of our animals. In some cases the vesicle ring does not completely form around the crust, so that at the height of the process the crust may be bordered in part by elevated and hyperæmic skin. In other lesions a vesicle may not at any time become recognizable to the naked eye. A variation which is not uncommon is for the lesion to undergo typical evolution, but for the different phases to be more or less delayed or accelerated. For example, a vesicular ring may be distinguished after 3 days or it may not be evident until after 6 days. In some lesions the process appears to start from a number of separate points instead of spreading symmetrically from the scratch.

The typical process is considerably modified by the character of the skin at the site of inoculation. Thus, on the thick and hairy skin of the back the several zones of the lesion, which are clear enough in an inoculation on the abdomen, are not distinguishable. The same phenomenon is seen when the skin of the tail is inoculated.

(b) *The occurrence and macroscopic appearance of the exanthem.*—In 65 monkeys inoculated on the skin of the abdomen with variola virus, in one form or another, a general exanthem was noted in 50 (77 per cent). In the animals in which vesicle contents was used for inoculation the exanthem occurred in 32 out of 40 (80 per cent). The exanthem was first noted on the seventh day of the disease in 7 animals, on the eighth day in 26, on the ninth day in 14, and on the tenth day in 3. When vesicle contents was used for the inoculation, the exanthem was first noted on the seventh day of the disease 5 times, on the eighth day 18 times, on the ninth day 8 times, and on the tenth day once.

The extent of the exanthem varied greatly. In some animals only 1 typical lesion was present, while in others over 100 were found.

The distribution of the exanthem showed a partiality for certain regions. The face was most often the site of an eruption. Elsewhere, roughly in the order of frequency, it was present upon the wrists, the scrotum of the male, the region about the anus and base of the tail, on the palms of the hands and the soles of the feet, and on the inner aspect of the arms and thighs. The eruption seemed to avoid the trunk and the outer hairy surfaces of the limbs.

The evolution of the exanthem was rather constant. The first appearance of the eruption was as minute, pink papules, rarely exceeding 1 millimeter in diameter. On the next day this papule was larger, often measuring 2 millimeters and showing a vesicular structure. In the majority of cases the fluid contents of the lesion became cloudy on the next day, and the lesion was completely dried in another 24 hours. The exanthem, therefore, has its complete evolution in about 4 days. In some animals the lesions pass through a longer period of development. In such lesions the papular, vesicular, and pustular stages could be recognized with as much certainty as in the primary lesions or as in the eruptive lesions of variola vera of man. The phenomenon was noted of the lesions appearing first on the face and later on other parts of the body and of their drying up in the order of their appearance.

The constitutional reaction, aside from the temperature, which might be taken as an indicator of the general reaction of the inoculated animal, showed little of a definite nature. At a time when the primary lesion is in its active stage, about the sixth to the eighth day of the disease, the animal sometimes shows some degree of anorexia and a tendency to droop, but at no time does it present such a constitutional reaction as is seen in the case of *variola vera*, of even moderate severity, in man.

The temperature reaction in *variola inoculata* in the monkey, differing from the same condition in its companion disease, vaccinia, presents a very definite curve. A comparison of the temperature charts of 20 animals, inoculated on the skin of the abdomen with vesicle contents, shows a marked rise in the body temperature on the sixth day of the disease in 14, on the seventh day of the disease in 2, and an indefinite one in 4. In only 3 of the animals was there no distinct elevation. This onset temperature may reach 41° C. In most cases the fever declines by lysis. The temperature reaction precedes the appearance of the exanthem by 24 to 48 hours.

The lymph nodes, which are interposed between the area of skin on which the primary lesion develops and the main lymph trunks, show a definite reaction. On the fourth or fifth day of the disease they are increased in size. This tumefaction increases and may, when the inoculation is on the abdomen, result in an enlargement of the individual nodes in the axilla to a diameter of 1 centimeter or more. At the time

of the greatest swelling the nodes are markedly tender. After the ninth or tenth day the nodes become smaller but remain firmer than normally for a considerable length of time.

The viscera of the animals killed during the disease showed no macroscopic lesions. Particular attention was paid to the bone marrow and testes, as these organs are the site of the only specific visceral lesion in *variola vera* in man.

(c) *Inoculations of the skin with variola disks.*—The inoculations in this series of animals resulted in a primary lesion which conformed to the type described above, save that the whole process was retarded in its evolution. However, the occurrence of the exanthem is in sharp contrast to that on the animals inoculated with the contents of the variola vesicle. The exanthem occurred in only 3 of the 5 animals, appearing in one on the eighth day, in the other 2 on the ninth day of the disease. In these 3 animals a total of only 7 eruptive lesions were found. The exanthem passed through its evolution rapidly and the individual lesions were small. The temperature reaction was like that in the preceding experiments in 3 of the animals, while in 2 it was indefinite. In other respects the results were similar to those in which the contents of the variola vesicle was used for inoculation.

(d) *Inoculation of the skin with dried pustule contents.*—In both animals of this series the primary lesion was typical in its development but somewhat delayed in its evolution. No exanthem was observed. The temperature reaction was the same as in the animals inoculated with the contents of the variola vesicle.

(e) *Inoculation of Macacus nemestrinus.*—In this monkey there is considerable variation from the type of the disease seen in *Macacus cynomologus*. This difference is principally shown in the evolution of the primary lesion. In this species the vesiculation of the lesion is much less definite, and there is an exaggeration of the cedema beneath it. The exanthem differed in no way from that observed in the Philippine monkey. The temperature reaction was less definite. In other respects the results of the inoculations were similar.

2. (a) *Histological examination of the primary lesions.*—The evolution of the lesion at the site of inoculation is characterized by a combination of degenerative, exudative, and reparative processes. Some one of these phenomena dominate the picture at different stages of the lesion, and in these various cell types figure.

The earlier lesions collected during the first, second, and third days of the disease primarily present the process of repair of a simple wound of the skin, but to this is added a change in the epithelial cells which border the incision. In these cells some degree of swelling and of degeneration is evident, although the usual reparative power of the *rete* is retained in sufficient degree to bridge the defect caused by the inoculation during the third day of the experiment. The cytoplasmic phases

of *Cytoryctes variolæ* are found in the cells of the *rete* during these early stages of the lesion.

From the third day of the disease onward the picture becomes more complicated. The degeneration of the epithelial cells is quite extensive and is evident for some distance from the line of inoculation. This degeneration is preceded by more or less swelling of the individual cells and some, though not wide spread, proliferation. The thickening of the epidermis about the inoculation is in the main due to the former process. Concomitant with the appearance of these phenomena we find fluid collected between the epithelial cells which finally leads to the formation of definite cavities, the vesicular space being partly contributed to by the solution of the swollen and degenerated epithelial cells. The polymorphonuclear leucocytes pass into the fluid of this vesicle, and their increasing numbers finally give to the lesion the macroscopic character which we designate as a pustule. While this vesiculation has been in progress, the crust which originated in the inspissated exudate in the inoculation scratch increases in extent and in thickness. The degeneration of the epithelial cells about the lesion is most marked where they form the floor of the vesicle, and becomes less and less as we pass to the peripheral portions of the lesion. Similar cell changes are found in the sheaths of the hair follicles close to the lesion.

While the vesicle has been forming in the epidermis, a reaction in the corium has become manifest. The first change, besides the collection of polymorphonuclear leucocytes in the vessels and their migration toward the vesicle, is enlargement and proliferation of the endothelial cells of the lymphatics and blood vessels. The connective-tissue cells in the corium undergo similar changes. Later in the disease a definite œdema of the corium and of the adjacent subcutaneous tissue is present, and an area of necrosis can be made out beneath the center of the lesion. Associated with this œdema and necrosis there is a large increase in the number of endothelial cells. These cells appear not only in and around the lymph vessels and capillaries but are found in the surrounding tissue; they contain cytoplasmic phases of *Cytoryctes variolæ* both when in situ on the walls of capillaries and when free in the tissue. Mitoses in these cells are frequent and some are met with containing two nuclei. The phagocytic properties of these cells are shown by their including leucocytes and other cells. Lymphoid and plasma cells, together with a certain number of eosinophile leucocytes are found in the corium at this time.

During the later stages of the lesion the process of repair dominates the picture. The epithelium grows in from the sides and up from the hair sheaths and finally closes the defect caused by the variolous process. In the corium the solution of the necrotic tissue and the new formation of blood vessels and connective tissue shows repair to be active.

(b) *The exanthem.*—In the development of the exanthem, the process seen in the epithelium agrees with that found in the primary lesion.



However, in the corium the process of oedema and necrosis is lacking, but there is some degree of cell reaction evidenced by the enlargement and proliferation of endothelial cells. The emigration of polymorphonuclear leucocytes is a prominent feature of the process.

(c) The lymph nodes associated with the primary lesion show a marked reaction, consisting in proliferation of endothelial cells in the sinuses and in the follicles and in the active phagocytic properties of these cells. The presence of red blood corpuscles and of polymorphonuclear leucocytes in the sinuses and the small areas of hemorrhage in the follicles appear also to be a part of the process.

(d) The internal organs show nothing which can be interpreted as manifestations of the disease produced in the animal by the inoculation.

#### DISCUSSION.

We have seen that the inoculation of the skin of the monkey with variola virus brings about a disease which exhibits characters at once relating it to variola in man and to vaccinia in man and in animals. The disease which follows an inoculation of the skin of the abdomen of *Macacus cynomologus* with fresh variola virus consists essentially in—

- (1) The development of a lesion at the site of inoculation.
- (2) The appearance of a general cutaneous eruption of vesicular lesions.
- (3) The enlargement of lymph nodes in the axillæ and groin.
- (4) The constitutional reaction.

When we examine the primary lesion microscopically we find it to be a self-limiting process which passes through certain definite phases which are reflected in the gross appearances and are described as vesiculation, pustulation, and crusting.

When we turn to the lesions of the exanthem we find that the characteristic phases of the primary lesion are produced in them.

As we have said, the disease presents a series of characteristic phenomena, and when a number of animals are simultaneously inoculated we see that these phenomena bear a definite time relation to one another. If we emphasize this time element we find that the phenomena of the disease occur as follows.

The evolution of the primary lesion covers a period of about 14 days from the time of inoculation. The first portion of this period comprises the active evolution of the local process. This period of active growth terminates on about the seventh day of the disease. It is difficult exactly to say when the lesions stop developing, but after combining various observations, this date is selected as the probable average time for the acme of the active evolution of the primary lesion. During the remainder of the period the phenomena of repair are dominant in the lesion.

The general exanthem appears in the majority of cases on the eighth

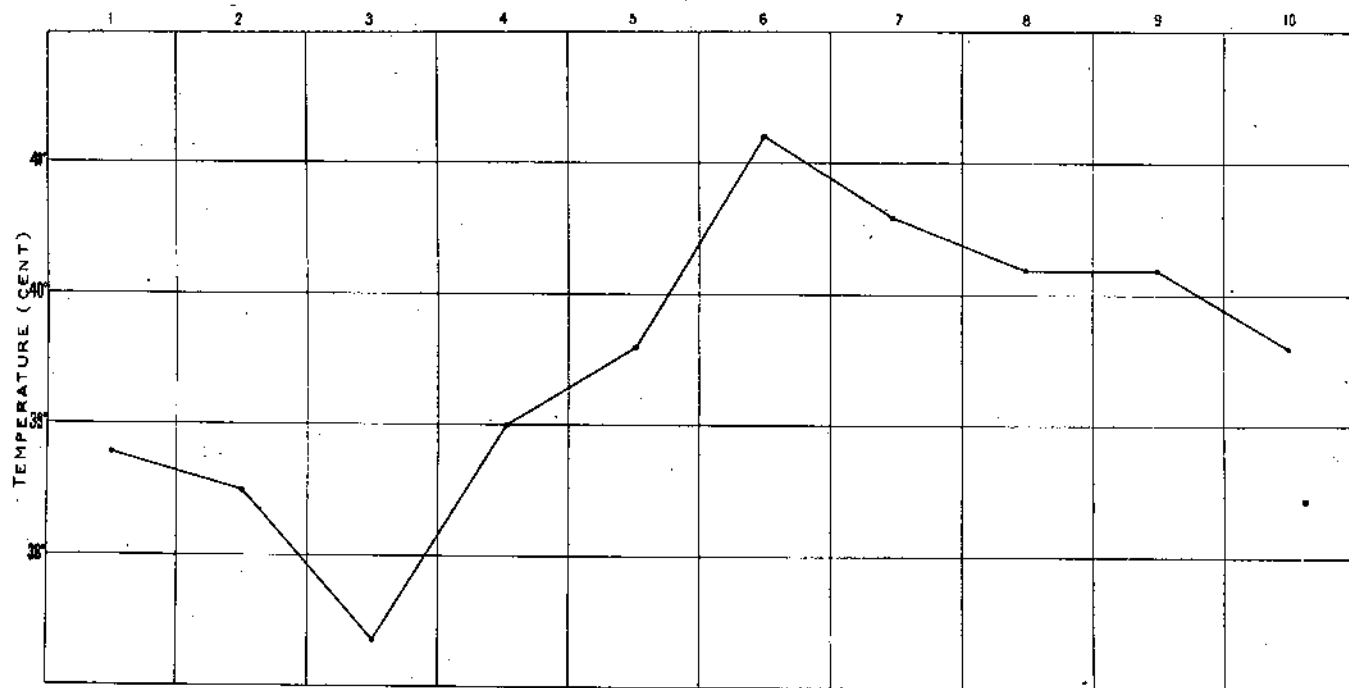
day of the disease and has a duration of about 5 days, the first 2 of which are employed in growth and the last 2 in healing.

The lymph nodes show an enlargement on the fourth day of the disease and are always markedly enlarged on the fifth. They decrease in size during the crusting of the primary lesion, but they are firmer than normal for a considerable time after the healing of the process.

The constitutional reaction occurs at about the height of the active phase of the primary lesion—that is, on the sixth and seventh day of the disease. In a great majority of cases the temperature reaction begins on the sixth day of the disease and persists for 2 or 3 days. It is difficult to say just how long the fever lasts, as the decline is by lysis, but it is almost always within normal limits by the twelfth day.

FIG. 2. TEMPERATURE REACTION.

DAYS OF DISEASE:



No. 118. VARIOLA INOCULATA IN THE MONKEY.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the disease which follows inoculation of that animal with vaccine virus we see at once that the processes are closely related. They are similar in that a self-limiting lesion appears at the site of inoculation, that the development of this lesion is associated with more or less constitutional reaction, and that certain lymph nodes become enlarged. The disease produced by inoculation with variola virus differs from vaccinia in that the primary lesion is usually followed by a general cutaneous eruption of lesions similar in many respects to the primary lesion and in that the temperature reaction is more abrupt in its onset and more intense.

If we compare the disease produced in the monkey by cutaneous inoculation with variola virus with the various manifestations of variola in man, we see that the disease produced is more like *variola inoculata* than any of the other forms. In fact the only differences which we find between the two lie in the time of occurrence of the general exanthem and in the duration of the temperature reaction. Thus, in the monkey we have the exanthem appearing on the eighth day of the disease, the temperature, which appeared on the sixth day, quickly falling by lysis, while in *variola inoculata* in man the exanthem appears on the eleventh and the temperature persists from the seventh to the ninth day. The development of the primary lesion and of the exanthem are practically the same in both.

When we compare *variola inoculata* in the monkey with *variola vera* in man we find that only certain characteristics of the disease type are held in common.

Different forms of virus, such as vesicle contents, pustule contents, and disk, were used for inoculations. On the skin, all these forms of virus produced typical primary lesions. However, it is to be noted that the nature of the contagium seemed to have an influence upon the occurrence and extent of the exanthem. We feel that these differences are explainable upon physical grounds and have to do with the reaction of the virus to external conditions rather than to any difference in the virus which is inherent to different ages of the lesion from which it is collected. This question will be taken up in another section of this article.

In the course of our experiments monkeys of all ages and of both sexes were employed. We did not observe any difference in the reactions of these animals which could be attributed to these factors. The general physical condition of the animal did not seem to affect the results of the inoculations.

The histological study of the specific lesions and of the viscera in *variola inoculata* in the monkey adds some details to our picture of the disease. We see that the specific lesions are similar in most respects to the vaccine lesions of man and of animals, and to the lesions of the

exanthem of *variola vera* in man. As has been pointed out,<sup>6</sup> the specific lesions of *variola inoculata* in the monkey differ from the lesions of the exanthem in *variola vera* in man in the greater prominence of the polynuclear leucocytes in the former. The primary lesions of *variola inoculata* in the monkey differ from the vaccine lesions of that animal in the extent and character of the process in the corium beneath the lesion, it being more intense in *variola inoculata*.

It is evident that the lymph spaces of the skin are flooded with virus at the time of the inoculation, and this fact may be a factor in the early development of the exanthem in the inoculated disease. From the fact that cytocytes are demonstrable in the endothelial cells of the capillaries in the corium beneath a primary lesion of five days' duration, it seems probable that at the time of inoculation, cells of this type become infected. Such infected cells in the lymph spaces or in the capillaries might easily be swept away in the circulation, and lodging in skin capillaries become the focus for an exanthem.

The absence of focal lesions in the bone marrow and testicles in *variola inoculata* in the monkey emphasizes the difference already mentioned between the disease experimentally produced in the monkey and *variola vera* in man.

#### CONCLUSIONS.

(1) Inoculation of the skin of the monkey (*M. cynomologus* and *M. nemestrinus*) with variola virus produces a disease in which all the essential characteristics are identical with those of *variola inoculata* in man.

(2) *Variola inoculata* in the monkey differs from *variola inoculata* in man in that the fever has a shorter duration and the exanthem appears at an earlier date.

(3) *Variola inoculata* in the monkey is as distinct a clinical entity as is *variola inoculata* in man.

(4) *Cytocytes variolæ* are found in the endothelial cells of the capillaries in the corium beneath the primary lesion of *variola inoculata*.

#### 2. VARIOLA INOCULATA IN THE ORANG-UTAN.

*Introduction.*—The experiments here reported were performed to determine the reaction of the orang-utan to inoculation with variola virus and to obtain material for the microscopic study of the specific lesions and of the morphology of the causative organism in this species of animal. Four orang-utans were procured for this work, but unfortunately only two of them survived long enough for the experiments to be carried out. These experiments are the first in which anthropoid apes have been used as the experimental animal in a study of small pox. The systematic position of the orang-utan in the animal kingdom makes it of peculiar

<sup>6</sup>Magrath and Brinckerhoff: *Jour. Med. Research*, 11, 230.

value in the comparative study of such a disease, it being susceptible both to diseases peculiar to animals (hæmorrhagic septicæmia) and to those common to man and to animals (amoebic dysentery). This makes the animal an ideal one for bridging the gap between the monkey and man in the study of the reactions of various mammalian hosts to a given disease-producing parasite. We regret that owing to the difficulty of acclimating these animals our data are not as abundant as might be desired.

*Technique.*—Two young female orang-utans were inoculated on the skin of the abdomen with variola virus in the same way as were the monkeys in the work the details of which have already been described. The animals were observed daily and the evolution of the lesion at the site of the inoculation and the constitutional reaction was recorded. Material for the histological examination of the specific lesions was collected. The details of the experiments are as follows:

No. 197. Young female orang-utan. The animal was first exposed to small-pox fomites. The results of this experiment will be considered in another article.

Twenty-one days after the exposure, the animal was inoculated in 12 places on the abdomen with virus No. 252. Forty-eight hours after inoculation there was slight elevation about the scratches. The deep pigmentation of the skin made it impossible to tell whether or not hyperæmia was present.

Five days. Along each line of inoculation there was a narrow, yellow crust seated upon a vague elevation. On gentle pressure turbid fluid exuded from beneath the crust. A small amount of this fluid was used to inoculate a Philippine monkey on the skin of the abdomen. This animal developed a typical pock at the site of inoculation and subsequently a profuse general exanthem.

Six days. The primary lesions were somewhat more prominent and the whole of the area beneath them was indurated. Axillary lymph nodes enlarged, firm and tender.

Seven days. The animal had scratched the lesions and they presented shallow ulcerations with ragged and often bleeding edges. The subcutaneous œdema had increased in extent, causing a brawny induration over the whole field of inoculation. The animal died during the night of an intercurrent infection.

No. 198. Young female orang-utan. Inoculated in 12 places on the skin of the abdomen with virus No. 199. After 24 hours slight elevation was apparent along the lines of inoculation. Body temperature 36°.1 C.

Four days. A narrow crust marked the scratch and was bordered by a distinct elevation. No change of color could be distinguished on account of the deep pigmentation of the skin. Body temperature 35°.9 C.

Five days. The elevation of the lesions had increased and they had become circumscribed. Body temperature 36°.6 C.

Six days. The zone of elevation was distinctly vesicular near the crust and clear serum oozed out on gentle pressure. Body temperature 36° C.

Seven days. Lesions had increased somewhat in extent and the crust had spread. Body temperature 35°.7 C.

Eight days. Animal found dead in cage early in the morning. *Rigor mortis* present, body still warm. Autopsy at once. *Skin.*—The inoculation sites present crusts, about which is a shallow cavity filled with turbid fluid. This vesicle appears to lie between the true skin and the epidermis. The border of the lesion shows some thickening of the skin, but this is not nearly so marked as at a corre-

sponding period in the primary lesion of *variola inoculata* in the monkey. No evidence of a general exanthem is present. Axillary lymph nodes enlarged, measuring 1 to 1.5 centimeters in diameter, deep-red in color, and on section rather dry. Peritoneal cavity contains one liter of clear, straw-colored fluid. Surfaces of normal color and texture. Pleural and pericardial cavities normal. *Heart*.—Valves and cavities normal; myocardium red-brown in color and of firm consistency. *Lungs*.—Normal. *Spleen*.—Capsule smooth, purple, on section pulp rather dry, color deep red-brown. Malpighian bodies and trabeculae not prominent. *Liver*.—General surface smooth and yellow-brown in color. On section, markings distinct and consistency normal. *Pancreas*.—Normal. *Gastro-enteric tract*.—Stomach normal. The mucosa of the cœcum and of the colon presents numerous punctate hemorrhages. *Kidneys*.—Capsule strips readily from a smooth yellow-brown surface. On section general color opaque yellowish-brown with irregular areas of injection in the cortex. Glomeruli visible as bright, red points. Genital organs and bladder normal. Bone marrow of femur deep red, homogeneous, and of firm consistency. Brain and meninges normal. Smears from the heart's blood show immense numbers of small, short, bacilli, which take a polar stain with Loeffler's methylene blue.

*Bacteriological examination*.—Cultures from the heart's blood, the liver, and the spleen yield an organism which was identified by Dr. W. B. Wherry, bacteriologist of the Biological Laboratory, Bureau of Science, as belonging to the group of organisms causing hemorrhagic septicæmia in animals.

*Histological examination*.—Primary lesion. Six days. The point of inoculation is marked by a complete destruction of the epithelium. The material present here consists of a lamella of cornified epithelium which fuses with a more or less homogeneous crust in which cell elements can occasionally be recognized. The upper layers of the corium at this place are more or less extensively necrosed, the connective-tissue fibers are swollen and fused with one another and with the crust. Cell detritus is scattered through this tissue. A fibrin network lies in the lacunæ of the corium in this region. On either side of the line of inoculation, immediately adjoining the crust, the cells of the *rete* are recognizable but are very much degenerated. Between these degenerated cells and the cornified layer are finely granular areas of the size of epithelial cells, each outlined by a membrane, but not containing recognizable cell structure. Polymorphonuclear leucocytes are present in this tissue, their nuclei often fragmented or show other signs of degeneration. As we pass outward from the line of inoculation the degenerated *rete* splits horizontally, one layer more or less imperfect following along the surface of the corium, while the other, still merging superficially with the degenerated strata above, curves up to form an almost complete band across the vesicle. Between this superficial layer of the *rete* and the corium is a lenticular cavity filled with a fine, granular material and containing a fibrin network. At the outer limits of the vesicle the cornified layer sweeps downward to merge with the thickened epidermis which marks the outer limits of the lesion. The layer of *rete* which forms the floor of the vesicle likewise runs into this thickened region. Beyond the main vesicle small cavities are to be seen in the middle layers of the epidermis. Some of these are evidently formed by hydropic degeneration of the cells, the cell membranes persisting as partitions across the cavity. In others these partitions have disappeared and the process seems in part due to an accumulation of fluid within and between these cell cavities. On the other side of the line of inoculation vesicle formation is evident for a considerable distance. Here, however, the *rete* remains intact and the collection of fluid is between and within the cells of the *stratum granulosum* and *stratum spinosum*. In places this vesicle is traversed by more or less vertical partitions composed of compressed epithelial cells.

The reaction of the corium is evidenced by some infiltration, with polymorpho-nuclear leucocytes and a certain degree of swelling of the endothelial cells lining the blood vessels and lymphatics of the corium adjacent to the site of the inoculation. Immediately beneath the lesion endothelial cells of lymphatics and of capillaries were found which contained in their protoplasm cytoplasmic phases of *Cytoryctes variolæ*.

The epithelial cells of the lesions contain many stages of the parasite. Both the cytoplasmic and the nuclear phases were well represented.

Seven and eight days. Lesions of these durations showed the same characteristics as did that described above. The vesicle becomes more extensive and the necrotic area beneath the crust larger. The coalescence of the lateral vesicles often left single cells or islands of cells in a fair state of preservation, which were to be found in various stages of agglomeration on the way to the formation of trabeculae, or partitions in the large vesicle.

Viscera. Histological study of the internal organs did not reveal any lesions of a variolous nature. All through the organs, wherever blood vessels were cut, short bacilli having the morphology and staining peculiarities of the bacillus of hemorrhagic septicæmia were readily demonstrable.

#### SUMMARY.

The evolution of the primary lesion at the site of inoculation with variola virus in the orang-utan is similar to that which follows the inoculation in the monkey and in man. The thickness and the deep pigmentation of the skin of this animal rendered the appearance less characteristic to the naked eye than in *Macacus cynomologus*. The death of the animals, of intercurrent disease, before a general exanthem might be expected to develop, deprived us of data upon this point. Both animals showed a marked constitutional reaction, but this, because of the existence of an intercurrent disease, can not be interpreted as resulting from the smallpox process.

The histological study of the primary lesions and of the viscera of the orang-utan inoculated with variola virus shows the process to be essentially similar to that which follows inoculation of the monkey with the same virus. There seems to be some difference in the degree of reaction in the corium beneath the primary lesion, it being notably less in the orang-utan. The histogenesis of the cutaneous vesicle is similar and we note the absence, as in the monkey, of focal lesions in the bone marrow. The most striking thing about the primary lesions seems to be their richness in nuclear forms of cytoryctes. This matter will be taken up in detail in another paper and so will not here be further commented upon.

#### CONCLUSIONS.

- (1) The orang-utan is susceptible to *variola inoculata*.
- (2) The evolution of the specific lesion at the site of inoculation is comparable with that which follows similar inoculations in the monkey (*M. cynomologus* and *M. nemestrinus*).
- (3) The primary lesions of *variola inoculata* in the orang-utan stand closer to the cutaneous lesions of *variola vera* in man than do the primary



cutaneous lesions of *variola inoculata* in the monkey in respect to richness in forms of *Cytoryctes variolæ*, and particularly in the number of nuclear forms present.

### 3. VARIOLOUS KERATITIS IN *MACACUS CYNOMOLOGUS*:

*Introduction.*—In this section will be considered the results of a series of inoculations of the cornea of the Philippine monkey with variola virus. These experiments were undertaken to determine what variations in the type of disease might result from a change in the locus of inoculation; they also yielded material for the histological examination of the specific lesion in tissue which is ideal for the study of cell changes owing to perfect preservation and the relative simplicity of the tissue elements.

*Technique.*—The methods of inoculation and of observation were the same as those described in the section upon vaccinal keratitis in the monkey.

*Details of experiments.*—Eighteen animals were employed in this series, of which the following experiments are selected to be given in detail:

No. 223. Monkey inoculated on both corneas with virus No. 200. After 48 hours slight unevenness was apparent along the line of inoculation. After 96 hours a minute defect in the corneal epithelium was present in the inoculated area. Chloroformed after 8 days. The cornea presents a small defect in the epithelium with very slight unevenness about it. No photophobia was observed. There was no general exanthem.

*Histological examination* showed proliferation of the epithelium about the inoculated wound and the presence of cytoryctes.

No. 224. Monkey was inoculated on the cornea with virus No. 200. After 48 hours the cornea was slightly uneven along the line of inoculation. Photophobia and haziness of the cornea was observed after 96 hours. Conjunctivitis was not present. On the sixth day of the disease a general exanthem consisting of 5 small vesicles was observed on the face and extremities. On the next day 5 new vesicles appeared. The animal was chloroformed on the seventh day. At autopsy no evidence of an initial lesion other than that on the cornea was demonstrable.

*Histological examination.* At the site of inoculation enormous numbers of cytoryctes were present in the epithelial cells. No leucocytes were found in the lesion or in the corneal substance about it.

### SUMMARY.

The inoculation of the cornea with vesicle contents was followed by the development of a lesion which had much in common with that which follows a similar inoculation on the rabbit. The following summary of the macroscopic appearances is based upon the observation of 18 experiments similar to those given above.

After 24 hours there is some roughening of the surface along the line of inoculation. After 48 hours there follows more or less loss of substance at the site of the inoculation incision. This loss of substance is

not so great as in vaccination of the cornea. When pyogenic infection does not complicate the process there is no opacity of the cornea or conjunctivitis, and the lesions heal after a variable period. In 2 animals, which were allowed to survive long enough for the eruption to appear, an exanthem was seen on the sixth and seventh days of the disease. The evolution and extent of the exanthem was like that following the skin inoculation. Another animal, kept under observation for a long period, did not develop an exanthem.

Histological examination shows that the process at the site of inoculation with variola virus on the cornea of the monkey consists primarily in degeneration and in proliferation of the epithelial cells. Seventy-two hours after the inoculation when the process is at its height the line of inoculation is marked by a defect in the epithelium, below which there may be a slight destruction of the corneal substance. The epithelial cells about this defect may be swollen and separated one from another, and show various degrees of degeneration. As we pass from the center of the lesion toward the periphery we find the epithelium much thickened. This increase in the thickness is in part due to swelling of the individual cells, shown particularly by those of the lower layer, which appear pale and assume a cuboidal or cylindrical form, and in part to an increase in the number of cells. At the point of greatest thickening the epithelium may measure twice its normal depth. In lesions of greater duration the degeneration of the individual cells and collection of fluid between the cells may occasionally result in the formation of minute cavities in the thickened epithelium, which are analogous to the vesicle of the specific skin lesion.

Polynuclear leucocytes do not form a prominent feature of the corneal lesions. In many sections a prolonged search is necessary to find a single cell of this type. When the inoculation wound penetrates the corneal substance polynuclear leucocytes are more numerous. The paucity of these cells in the lesion is in strong contrast with the condition in the cutaneous smallpox lesions in the monkey.

*Cytoryctes variolæ* were present in all the lesions examined. Their morphology and staining reactions were identical with those found in the skin lesions. No nuclear phases were present. The parasites were found as early as 18 hours after the inoculation and persisted through the eleventh day, that date being the last on which a microscopic examination was made.

#### DISCUSSION.

When we compare the lesion produced on the cornea of the monkey by inoculation with variola virus with vaccinal keratitis in the same animal we see at once that we have to do with a similar process. The only striking difference which these two lesions present is that in the variolous keratitis there is less exudation and the epithelium of the lesion does not become detached and cause a large superficial defect as in the

vaccinial lesion. In this respect variolous keratitis in the monkey approaches more nearly to vaccinial or variolous keratitis in the rabbit than does vaccinial keratitis in the monkey.

The small part played by the polynuclear leucocyte in the variolous keratitis contrasts strongly with the prominence of this cell in the specific cutaneous lesions of the disease in the monkey. It seems probable that in the skin inoculation the presence of a large number of these cells is conditioned by the destruction of tissue incident to the inoculation and that they continue to be attracted, not so much by the variola organism or its products as by the presence of substances set free in the process of cell destruction incident to the activity of the parasite and the other organisms which gain access to the lesion. As all such degeneration products are retained at the site of inoculation by the crust, in the case of a cutaneous lesion, while they readily escape in the case of the corneal one, it is easy to understand why in the former case large numbers of leucocytes pass from the vessels to the lesion.

The absence of nuclear phases of *cytocytes variolæ* in the corneal lesion was disappointing. It is possible that there is some inherent difference in the cells of the cornea which makes them unsuitable for the development of the parasite beyond the cytoplasmic stage, though we are inclined to regard the absence of nuclear forms in these lesions as being due to the action of physical factors. In a typical corneal lesion the cells are probably cast off from the surface before the nuclear forms are produced. It is possible that oblique incisions into the corneal substance, in which islands of epithelial cells would be retained, might show a development of the nuclear forms of the parasite.

In a previous section we have shown that the endothelial cells of capillaries beneath the primary skin lesions of *variola inoculata* are invaded by cytocytes. From this we have been led to believe that some such process is involved in the dissemination of the organisms to form the exanthem. From the considerations of the time elements it seems probable that such infected endothelial cells are set free at an earlier stage in the disease than the demonstration of the infected endothelial cells beneath the primary lesion. The fact that an exanthem follows the development of a lesion in the nonvascular cornea, where extension of the process to blood vessels does not occur, suggests that the distribution of the organism, if brought about through the intermediation of endothelial cells, is due to an infection of the cells which line lymph channels.

#### CONCLUSIONS.

(1) Inoculation of the cornea of the monkey (*M. cynomologus*) with variola virus produces a specific lesion characterized by swelling, proliferation, and varying degrees of degeneration of the epithelial cells.

(2) The lesion is similar to that produced by inoculation of the cornea of the rabbit with vaccine or with variola virus.

(3) The lesion results in less destruction of the corneal epithelium than follows similar inoculations of the cornea of the monkey with vaccine virus.

(4) The lesion on the cornea differs from the variolous lesion on the skin of the monkey in that exudation does not play as prominent a part and that true vesicle formation does not occur.

(5) *Cytoryctes variolæ* are present in the lesion up to 11 days after the inoculation, but nuclear forms of the parasite are not found.

(6) A variolous lesion on the cornea of the monkey may be followed by a general exanthem which appears on the same day as after skin inoculation. We therefore identify the disease produced in the monkey by variolation on the cornea as *variola inoculata*.

#### 4. VARIOLA INOCULATA FOLLOWING INOCULATION OF THE MUCOUS MEMBRANE OF THE MONKEY.

*Introduction.*—In the preceding sections of this paper we have detailed the results which follow the inoculation of the skin and the cornea of the monkey with variola virus. We will present here a series of experiments which show the results of inoculation of that animal upon the mucous membrane of the nose, the lip, and the palate. The experiments also yielded material for the study of the variola organism and the histology of the specific lesion on the mucous membrane.

*Technique.*—The method of inoculation and of observation was the same as that followed in the series of inoculations of the mucous membranes of the monkey with vaccine virus.

*Clinical course of the disease.*—Twenty-nine animals were used in this series of inoculations, of which the following are selected to be described in detail:

No. 125. Adult male, *Macacus cynomolgus*. Inoculated on the left side of the nasal septum, on the inner side of the lower lip, and on the left palate with variola virus No. 167 (vesicle contents). Body temperature 40° C.

Twenty-four hours after inoculation a slight elevation is noted on the nasal septum. The lip and palate are negative. Body temperature 38°.8 C.

Forty-eight hours. On the lip a narrow white line surrounded by hyperæmic mucous membrane marks the site of inoculation. Nose and palate negative. Body temperature 40° C.

Three days. The nose shows considerable swelling of the septum, but no distinct lesion can be distinguished. The lip presents a white area, 1 × 3 millimeters, which is slightly elevated and has a translucent appearance. The palate shows a small, gray spot on the line of inoculation. Body temperature 40° C.

Four days. Nose negative. The lip shows a white, opaque area 1 × 3 millimeters, with a ragged elevated edge, about which the mucosa is distinctly reddened. The palate presents a white, slightly elevated area, 2 × 4 millimeters, the surface of which is unbroken. Body temperature 40° C.

Five days. Nose negative. The lip shows an opaque, white area, covered in part by the remains of the macerated epithelium, and surrounded by a dull pink elevated border. The palate presents an elevated area 2 × 4 millimeters in extent and of a gray-white color. About this are smaller similar spots. Body temperature 39°.5 C.

Six days. The nose presents an opaque area on the septum surrounded by a bright red areola. The lip is much swollen and the lesion shows an area of erosion surrounded by a ragged edge of elevated and hyperæmic mucous membrane. The lesions on the palate have increased somewhat in size and show a distinct red areola. Body temperature 40° 2 C.

Seven days. Considerable mucopurulent discharge from the nostril; swelling of the mucous membrane prevents inspection. Lesion on lip as before. The palate shows some increase in the size of the lesion. Body temperature 40° C.

Eight days. The swelling of the mucous membrane in the nose continues. On the lip the lesion presents as an ulceration with marked inflammatory reaction about it. On the palate the lesion shows no erosion and has ceased to spread. Body temperature 40° 4 C.

Nine days. Lesions as described yesterday. A general exanthem was carefully searched for but not found. Animal killed and autopsy done at once. No macroscopic evidence of disease except at the sites of inoculation.

No. 126. Half-grown male, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding animal. Body temperature 39° C.

After 24 hours the nose shows a delicate crust on a slightly elevated area of mucous membrane. On the lip a small lacerated wound marks the point of inoculation. The palate presents some reddening of the mucosa about the scratch. Body temperature 38° C.

Forty-eight hours. Lesions as above described. Body temperature 39° C.

Three days. In the nose the elevation about the crust has increased. The lip shows an elevated, opaque, white area, 3 × 4 millimeters, surrounded by a diffuse red flush. On the palate is a slightly elevated grayish area, 2 × 4 millimeters in extent, surrounded by hyperæmic mucosa. Body temperature 40° 4 C.

Four days. Nose as before, save that the elevation has taken on a gray tint. The lip presents a macerated appearance over the area occupied by the lesion. The lesion on the palate has increased in size. Body temperature 40° 5 C.

Five days. On the palate the lesion is eroded, otherwise there is no change. Body temperature 39° 5 C.

Six days. The nose presents a narrow crust on a pink elevation. The lesion on the lip shows signs of healing. Palate as before. The top of the tongue near its tip presents a gray elevated area, 2 millimeters across, surrounded by a pink areola. Lesions show no change since yesterday. Animal killed and autopsy at once made. Viscera show no macroscopic lesions. A single small vesicle outside the left eye is shown to be specific by microscopic examination. No other exanthem found.

No. 127. Young female, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature 39° 2 C.

Twenty-four hours after. Slight elevation and reddening at the points of inoculation. Body temperature 38° C.

Forty-eight hours. In the nose there is reddening of the mucosa on the septum. On the lip a yellow line surrounded by slightly elevated and pink mucosa marks the site of inoculation. Palate negative. Body temperature 39° C.

Three days. Nose negative. Lip shows a gray line on a white elevated area 2.5 × 5 millimeters, the edge of which is translucent. Palate presents a grayish-white line 3 millimeters long. Body temperature 39° 5 C.

Four days. Nose negative. The lip shows a gray-white area, 2 × 5 millimeters, with a pink edge and a granular surface. Palate as before. Body temperature 39° 5 C.

Five days. Nose negative. The lip is swollen and presents an erosion with a yellow surface and a thickened, pink edge 2 × 4 millimeters in extent. Palate

presents an eroded area  $2 \times 5$  millimeters, with a slightly depressed gray base and an elevated margin which is surrounded by reddened mucosa. Body temperature  $38^{\circ}.5$  C.

Six days. Lesions as before but somewhat increased in size. Body temperature  $39^{\circ}.5$  C.

Seven days. Nose presents a narrow crust about the orifice of the left nostril. The lip shows a large erosion 1 centimeter across, the base of which is covered by yellow, macerated material and is surrounded by an elevated, ragged, pink border. Lesion on palate has increased in size. Body temperature  $38^{\circ}.8$  C.

Eight days. Nose shows an eroded area extending from the septum out upon the surrounding skin. Lesion on lip as before. Palate presents a long, narrow ulceration  $2 \times 8$  millimeters in extent, with a white, elevated edge, and surrounded by a red areola. One small, pink, papular elevation on the inner side of each thigh and on right arm. Histological examination shows this lesion to be varicellous. Body temperature  $38^{\circ}.5$  C.

Animal killed; at autopsy organs appear normal.

No. 128. Full grown female, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding monkeys. Body temperature  $39^{\circ}$  C.

After 24 hours there is slight reddening of the mucosa about the scratches. Body temperature  $38^{\circ}.2$  C.

Forty-eight hours. Lesions as before. Body temperature  $38^{\circ}.2$  C.

Three days. The lip is swollen and presents a white area, 1 by 3 millimeters, slightly elevated and surrounded by a pink areola. Palate presents 2 yellow elevations 2 by 4 millimeters in extent with a red periphery. Body temperature  $39^{\circ}.5$  C.

Four days. Lesion on lip is excoriated. Palate as before, but lesions have spread somewhat. Body temperature  $39^{\circ}$  C.

Five days. Lip shows an eroded area with a gray base and a white, elevated edge, surrounded by reddened mucous membrane. The whole lower lip distended, swollen and indurated. Palate as before. Body temperature  $38^{\circ}.2$  C.

Six days. Swelling of the lip more marked. The base of the lesion is white and the edge ragged. A minute, pink papular elevation appears on the gum opposite the lesion. Probably an auto-inoculation. Palate as before. Body temperature  $39^{\circ}$  C.

Seven days. Lesions as described but somewhat increased in extent. Body temperature  $38^{\circ}.5$  C.

Eight days. From this time on the primary lesions healed without complication. A general exanthem was not found either before or after this date. Body temperature  $37^{\circ}.5$  C.

No. 129. Full grown male, *Macacus cynomologus*. Inoculated in the same way and with the same virus as the preceding animals. Body temperature  $38^{\circ}.5$  C.

Twenty-four hours after inoculation the lip and palate show a white line surrounded by hyperemic mucosa. Nose negative. Body temperature  $39^{\circ}.8$  C.

Forty-eight hours. Lip and palate show irregular elevation with opacity and a peripheral flush at the site of inoculation. Nose negative. Body temperature  $38^{\circ}.2$  C.

Three days. Lip presents a yellow, eroded area, 2 by 5 millimeters, surrounded by a pink areola. Palate shows an opaque, white area, 2 by 5 millimeters, surrounded by reddened mucosa. Body temperature  $39^{\circ}$  C.

Five days. Lesions on lip and palate somewhat increased in extent. The inoculation in the nose evidently did not take. Body temperature  $39^{\circ}.4$  C. Animal killed and autopsied at once. Viscera, present no macroscopic lesions.

A series of 10 monkeys were inoculated on the palate alone and a series of 9 upon the nasal mucosa alone. The results of the inoculations were similar to the experiments detailed above.

#### SUMMARY.

The lesion which develops on the inner side of the lip of the monkey following inoculation with vesicle contents presents the following macroscopic characters.

After 24 hours the site of inoculation shows, at most, a slight reddening of the mucous membrane about the scratch.

After 2 days a narrow, white line is seen, which is surrounded by a faint, red flush.

After 3 days there is a definite, opaque, white, area, 2 or more millimeters in extent, slightly elevated above the general surface. This area is more or less eroded and is surrounded by a distinct zone of hyperæmic mucous membrane.

After 4 days the opaque area is somewhat eroded and presents a shallow ulcer with an elevated, white, often sinous, edge, which is bordered externally by reddened mucosa.

From this time on the lesion presents the same characteristics, the only change being due to a gradual extension of the process. After 8 or 9 days the peripheral flush fades and healing begins. This process results in complete repair after about a week.

Inoculation of the palate causes a lesion similar to that following inoculation of the lip, and the lesion runs essentially the same course. In this situation the lesion is less apt to become eroded.

After inoculation of the mucous membrane of the nose it is difficult to follow the process from day to day, as at the time when the lesion is undergoing its active evolution the swelling of the mucous membrane which accompanies the process prevents inspection during life. From study of the site of inoculation in animals killed at various periods it is seen that the evolution of the lesion in the nose differs principally from those on the lip and palate in that there is less tendency to form an ulcer. When the inoculation is near the anterior nares the process tends to spread out on the skin about the nostril and then takes on the characteristics of a skin inoculation.

No notable constitutional reaction followed the inoculation of the mucous membrane. The temperature reaction was indefinite. A general exanthem was observed in 2 of the monkeys.

*Histology of the primary lesion.*—Lip: Sections from lesions collected 25 and 53 hours after inoculation show no evidence of a specific process. The defect in the epithelium caused by the inoculation has completely been repaired, and the only evidence of the wound is a small collection of fibrin and polynuclear leucocytes just beneath the epithelium.

Three days after inoculation the lesion shows specific characters and we find an area in which the epithelium presents a pathological change, and beneath this the tissue is infiltrated with polynuclear leucocytes. The changes in the epithelium consist in degeneration and disintegration of the epithelial cells, together with more or less accumulation of fluid in and between the cells. This accumulation of fluid occurs not only in the degenerating portion of the epithelium but also at the sides of the lesion. This process is similar to that seen in a skin inoculation of the same duration.

The degeneration of the epithelium is not uniform, and we find islands of comparatively normal cells in the midst of areas where the affinity of the nuclei of the epithelial cell for basic stains is lost. Polynuclear leucocytes are present in large numbers. The epithelium at the edge of the lesion is somewhat thickened, apparently as a result of the swelling of individual cells.

After 4 days the lesion shows the same characteristics, save that there is considerable loss of substance in the area of degenerated epithelium and the lesion is more extensive. At this time the reaction in the tissue beneath the lesion is well marked and proliferation and enlargement of the endothelial cells of the lymphatics and blood vessels is apparent. Many elements of the lymphoid and plasma cell series are present about the vessels beneath the lesion.

Five and 6 days after inoculation the lesions are similar in character to those just described, but the necrotic area becomes sharply limited and the inflammatory reaction beneath is more intense.

Lesions of 7 days' duration show evidence of beginning repair. The epithelium at the edge of the lesion is normal and the lesions consist of a sharply circumscribed ulceration, in the depths of which repair is active. Later lesions show the epithelium growing inward to close the defect, and new formed blood vessels and young connective-tissue cells are much in evidence in the tissue beneath.

In the lesions of 3, 4, 5, 6, and 7 days' duration epithelial cells containing cytoplasmic phases of *Cytoryctes variolæ* are of frequent occurrence. The earlier forms of the parasite occur at the margin of the lesion, the later ones nearer the center. Nuclear phases of the parasite are also found, but they occur later than the cytoplasmic phases.

*Nose.*—The primary lesions in the nose vary in character according to the locus of inoculation. When the incision is near the orifice of the nostril, on a stratified epithelium, the lesion conforms to the type described on the lip. When the lesion is higher up, upon a columnar epithelium, the process in the submucous tissue is most marked. In such a situation the bulk of the degenerated epithelial cells seem to be carried off almost at once, and we find but little thickening of the mucous layer. However, the submucous tissue shows accumulations of lymphoid and plasma cells, enlargement and proliferation of the endothelial cells of the



lymphatics and blood vessels, and a marked polynuclear leucocyte infiltration.

Both cytoplasmic and nuclear phases of *cytocyctes variola* are present in the epithelial cells. In one lesion, of 5 days' duration, an endothelial cell was found on the wall of a lymphatic, just beneath the epithelium, which contained a cytoplasmic form of the parasite.

*Palate.*—The histology of the primary lesion in this situation is similar to that seen in the lesion on the lip. A lymphatic with cytoplasmic forms of cytocyctes in the endothelial cells lining its wall was found beneath a lesion of 5 days' duration.

Histological examination of the viscera showed no lesions. The bone marrow and testicles were carefully examined for focal lesions, such as are found in these organs in *variola vera* in man, but none could be demonstrated.

#### DISCUSSION.

The disease produced by variolation of the monkey upon the mucous membrane of the lip, nose, and palate is characterized by the development of a self-limiting lesion at the site of inoculation, which may be followed by a general cutaneous exanthem and be associated with an indefinite constitutional reaction.

If we compare the initial lesion produced on the mucous membrane with that which follows a similar inoculation on the skin we see that the two processes are similar, in that they run a definite course and tend to heal after about the same interval of time. The microscopic study of these lesions shows them to be the result of similar cell changes, and in each the parasite, *cytocyctes variola*, is found associated with the process. The lesions differ in that on the mucous membrane the absence of crust prevents the development of a vesicle or pustule, although an accumulation of fluid between the cells is in evidence at certain stages of the lesion. The primary lesion on the mucous membrane, of 4 or 5 days' duration, simulates closely a skin lesion of the same duration which has lost its crust through rough manipulation. The primary lesion on the mucous membrane also differs from that upon the skin in that the process in the tissue beneath the lesion is more exudative and proliferative than necrotic.

When we compare the other manifestations of the disease following variolation of the mucous membrane with that following skin inoculation a decided difference is found. We see that a general exanthem is much less apt to follow the inoculation of the mucous membrane. Of 19 animals inoculated in the nose, on the lip, or on the palate only 2 showed a general exanthem. In both these animals the lesions of the eruption were few in number and required microscopic study for their positive diagnosis. The exanthem occurred in a trifle over 10 per cent of the animals. This is in sharp contrast with the occurrence of the exanthem

in *variola inoculata* from skin inoculation, where an eruption develops in from 70 to 80 per cent of the animals.

The constitutional reaction in monkeys variolated upon the mucous membrane differs in degree from that in those variolated upon the skin. We do not find such an abrupt elevation in the body temperature, and malaise and anorexia are absent. It seems reasonable to suppose that this relatively slight constitutional reaction is conditioned by the physical conditions at the *locus* of inoculation. The initial lesion on the mucous membrane is practically an open wound from the beginning, and consequently the products of cell destruction and any toxins produced by the specific organism can readily escape. The systemic absorption from lesions on the mucous membrane must be quantitatively much less than from the lesions upon the skin.

We are inclined to attribute the infrequency of the exanthem in this series of animals to physical conditions at the *locus* of the primary lesion. At the time of inoculation the amount of virus which enters and remains in the scratch must be notably less than when the skin is inoculated, owing to the fact that the surface inoculated is bathed with fluids and any exudation stream set up by the trauma of the inoculation would be assisted by the moist condition of the surface and the action of similar opposed surfaces to carry off the bulk of the virus. In these inoculations there can be no such flooding of the lymphatics about the scratch as must occur after similar inoculations of the skin.

The type of disease produced by variolation upon the mucous membrane conforms in general to that which follows inoculation of the skin. The nature of the primary lesion and the time of occurrence of the exanthem relate the disease at once to *variola inoculata*. The absence of focal lesions in the bone marrow and testicle differentiate the disease from *variola vera* in man.

#### CONCLUSIONS.

- (1) Inoculation of the mucous membrane of the lip, the nose, or the palate of the monkey (*M. cynomolgus*) with variola virus produces a disease which conforms to the type of *variola inoculata*.
- (2) The primary lesion on the mucous membrane is similar, cytologically and histologically, to that which follows variolation of the skin.
- (3) *Cytoryctes variolæ*, in both the protoplasmic and the nuclear phases, are present in the lesions.
- (4) Cytoplasmic forms of the parasite are found invading endothelial cells of lymphatics beneath the lesions of 5 days' duration.

#### 5. ON THE OCCURRENCE OF VARIOLA VERA IN MONKEYS AND IN THE ORANG-UTAN.

In the preceding sections we have shown that the monkey and the orang-utan react in a definite manner to inoculation with the virus of smallpox. This reaction consists in the development of a disease which

conforms closely to that type of smallpox in man which follows deliberate inoculation of the skin with variola virus. In short, we can produce in the monkey and in the orang-utan the homologue of human *variola inoculata*. We have also shown that if the epithelium of the cornea or of the mucous membrane of the nasal, buccal, or oral cavity be chosen as the *locus* of inoculation the same type of disease is produced. The experiments upon which this section are based were devised in an attempt to reproduce in the monkey and in the orang-utan a disease having the clinical features of *variola vera*. We have sought to attain this end by changing the *locus* of inoculation and by subjecting the animals to conditions in which man contracts the disease. The experiments which we have chosen to give in detail will be grouped according to the manner in which we have sought to bring about the infection.

(a) *Inoculation of the tracheal epithelium, through a tracheotomy wound, with the contents of the variola vesicle.*—These experiments were performed to determine if the course of the disease would be modified by the initial lesion being seated upon the columnar epithelium of the trachea. The generally accepted hypothesis of smallpox in man supposes a "protopustule" in the respiratory tract, and we proposed to deliberately produce such a lesion by direct inoculation with variola virus.

No. 144. Adult male, *M. cynomologus*. Monkey anesthetized with chloroform and a median incision made in the neck over the upper part of the trachea. Trachea exposed by blunt dissection. The upper ring of the trachea cut, and through the opening so made the epithelium on the posterior wall slightly scarified and inoculated with variola virus No. 167 (vesicle contents). The skin incision closed with silk sutures. After 4 days the operation wound showed a marked inflammatory reaction.

On the ninth day of the experiment 6 red, papular, elevations, 2 to 3 millimeters in diameter, were seen on the scrotum. On the next day papules and small vesicles were found on the face, trunk, and extremities. On the eleventh day of the experiment the lesions on the scrotum presented flat-topped, umbilicated vesicles with an opaque center and surrounded by a bright-red areola. The evolution of these lesions was comparable with that seen in the exanthem of a case of discrete *variola vera* in man. A distinct pit remained after the healing of the lesions. One lesion was found in the palm of the hand. The body temperature rose to 40° C on the sixth day of the experiment and remained elevated until the thirteenth day.

No. 139. Adult male, *M. cynomologus*. Inoculated in the same manner as the previous animal. A profuse general exanthem appeared on the seventh day of the experiment. Animal died on the ninth day. At autopsy extensive cellulitis of the neck about the operation wound. Smears from this region show many streptococci. *Mucous membranes.*—In cheek pouches about a dozen opaque, elevated, sharply circumscribed lesions, 3 to 4 millimeters in diameter and 1 millimeter high. One similar lesion on under surface of tongue. Similar though smaller lesions are found scattered over the surface of the œsophagus, some of which are eroded. In the trachea at the site of inoculation is a red, slightly elevated area, due apparently to thickening of the epithelium. *Viscera.*—No macroscopic lesions found. *Skin.*—Vesicles and pustules, from 3 to 4 millimeters in diameter, are

found scattered over the face, trunk, and extremities. The exanthem consists of over 100 lesions.

*Histological examination.*—*Operation wound:* Subcutaneous tissue contains much fibrin and finely granular precipitate. There is considerable necrosis of the underlying muscle. Polymorphonuclear leucocytes in all stages of degeneration are present in large numbers. Many small blood vessels are thrombosed, and in them and in the surrounding tissue streptococci are demonstrable. The epithelium at the edge of the wound is much degenerated, but no evidence of a variolous process can be made out. However, cytoryctes were found in the cells of the thickened corium. *Trachea:* The epithelium is wanting in places and in other areas it is somewhat thickened. In many places the basement membrane is broken. The epithelial cells show various degenerations. In many groups of cells cytoplasmic phases of *Cytoryctes variolæ* are demonstrable. The vessels of the submucous tissue are much injected, and in one is a mass of fibrin in which are phagocytic cells containing streptococci. The tissue is infiltrated with polynuclear leucocytes. The endothelial cells of a capillary immediately below an area in which the epithelium shows cytoryctes are prominent and contain cytoplasmic phases of the parasite. *Skin:* The lesions of the exanthem present the characters which before have been described in similar lesions occurring in the course of *variola inoculata* in the monkey. *Mucous membrane:* The lesions in the cheek pouches consist of areas of degenerated epithelium in which groups of cells are stained red, the nuclei having lost their affinity for basic dyes. The protoplasm is finely granular, but the cell outlines are retained. Other cells are present whose staining properties are unimpaired, and in these are found various cytoplasmic phases of *Cytoryctes variolæ*. In the upper layers of the degenerated epithelium, particularly toward the edge of the lesion, the cells are separated by fluid. In some places the appearance is similar to that seen near the edge of a 4-day primary lesion in the skin. Polynuclear leucocytes are present in the epithelium and in the tissue beneath, although in the latter situation the reaction is much less intense than it is beneath a primary lesion of the mucous membrane. *Esophagus:*—Small areas are present in which the epithelial cells are swollen and their nuclei shrunken. Evidence can be made out of accumulation of fluid in and between the cells. Cytoplasmic forms of *Cytoryctes variolæ* are present in many of the cells. Comparatively few polynuclear leucocytes are present in the lesion, and there is practically no reaction in the tissue beneath. *Seminal vesicles:* Focal lesions are present in the septa of the tubules. The lesions consist of collections of degenerated cells and fibrin which lie in the connective tissue stalks of the septa. Associated with this there is more or less degeneration of the adjacent epithelial cells. A few polynuclear leucocytes are present. Many of the epithelial cells contain cytoplasmic phases of *Cytoryctes variolæ*. *Testicle:* Normal. *Bone marrow:* No focal lesions are demonstrable. The lung, liver, spleen, and kidney show no lesions.

*Summary.*—From observation of the trachea in animals killed at various times after the inoculation it was seen that a distinct process occurs at the site of inoculation. Our data on this point seems to us insufficient to form the basis of a description of the stages in the evolution of the specific lesion in this situation.

A general exanthem was observed in 3 of the 4 monkeys of this series. The single animal which did not show an eruption died on the eighth day of the experiment. The exanthem appeared on the seventh day in 1 animal, on the ninth day in the other 2. The exanthem was very extensive and in the 2 animals in which it appeared on the ninth day

of the disease it passed through an evolution similar to that of the eruption of *variola vera* in man.

The constitutional reaction was very marked, the animals showing anorexia and weakness during the active stage of the disease. This marked constitutional reaction may be, in part, explained by the intensity of the pyogenic process in the operation wound. The temperature reaction was similar to that seen after skin inoculation, showing a marked rise on the sixth or seventh day.

The histological examination demonstrates that the lesion at the site of inoculation and the lesion of the exanthem on the skin and the mucous membranes was variolous in type. In 1 animal focal lesions were demonstrated in the seminal vesicles and they were shown to be specific.

The method of inoculation used in this series of experiments was abandoned, as it was impossible to exclude a variolous infection of the operation wound. Obviously if this were to take place the experiments would be merely repetitions of the skin inoculation complicated by a coincident infection of the trachea.

(b) *Inoculation of the epithelium of the trachea, without tracheotomy, with the contents of the variola vesicle.*—These experiments were devised to overcome the difficulty experienced in the previous series resulting from infection of the tracheotomy wound. This series consists of experiments on 10 monkeys (*M. cynomologus*), of which 1 will be described in detail. Six animals were killed at various times after the inoculation to obtain material for histological study.

No. 339. Adult male, *M. cynomologus*. Monkey anesthetized with chloroform and a large glass tube, having a fire-burnished end, introduced into the larynx through the mouth. By way of this tube instruments were introduced to scratch the wall of the trachea and to inoculate it with the virus. Variola virus No. 328 was employed.

On the seventh day of the disease the body temperature rose to 41° C., and a pink, papular elevation 1 millimeter in diameter appeared on the scrotum. On the next day small vesicles, surrounded by a bright-red areola, were present upon the face, scrotum, hands, and feet. On the following day, the ninth of the experiment, new eruptive lesions were found on the face, the scrotum, and the palms and soles. Animal chloroformed and autopsied at once. Skin lesions as described above. The mucous membrane of the trachea is congested throughout and presents several opaque spots on which are minute granular elevations. The trachea and large bronchi contain much slimy mucus. The left lung presents an area of consolidation, 5 millimeters in diameter, about the primary bronchus. On section, this area is reddish-brown in color and finely granular. Other organs appear normal.

*Histological examination.*—The microscopic study of the organs from these monkeys yielded data upon the variolous lesion in situations not previously described. The following descriptions are selected as types of the lesions found:

No. 345. *Trachea.*—Lesion of 3 days' duration. The basement membrane is intact. There is no reaction in the submucous tissue. The epithelial cells in a small area, which can be included in a single field of the oil immersion lens, contain various stages of the cytoplasmic forms of *Cytoryctes variolæ*. Aside from the vacuole about the parasite these cells appear normal.

No. 348. *Trachea*.—Lesion of 6 days' duration. The epithelium is thickened in places and frequently tongue-like projections, composed of epithelial cells, project into the lumen. There is wide-spread infection of the epithelial cells with cytoplasmic phases of *Cytoryctes variolæ*. In places groups of epithelial cells have lost their selective staining affinity and are colored an even red. Occasionally there is evidence of the collection of fluid within and between the degenerated cells. Polynuclear leucocytes are numerous and are found in the epithelium as well as in the submucous tissue. In places there is necrosis and accumulation of fibrin in the submucous tissue, such areas occurring beneath breaks in the basement membrane. The endothelial cells of the lymphatics and blood vessels of the submucosa are prominent and frequently contain cytoplasmic forms of *Cytoryctes variolæ*. *Lung*, 6 days after inoculation. The section shows a large bronchus and the surrounding lung substance. The epithelium of the bronchus is much thickened at 2 points in its circumference. In these areas the cells are more or less swollen and degenerated. The majority of the cells contain cytoplasmic forms of *Cytoryctes variolæ*. Polynuclear leucocytes are present in large numbers in the lumen of the bronchus, in the epithelium, and in the peribronchial tissue. The air cells for a considerable distance from this bronchus are filled with cells, fibrin, and granular precipitate. The relative amount of each of these constituents varies in different air cells, but in general the granular precipitate predominates in those remote from the bronchus. The cellular elements present are in the main polynuclear leucocytes and epithelial cells. The latter are found free in the air cells and also attached to the walls. In the latter situation they are frequently cuboidal in form and lie one beside another. Mitoses are frequently present in these cells. Cytoplasmic phases of *Cytoryctes variolæ* are present in large numbers in the epithelial cells of the air spaces. The capillaries in the septa between the affected air cells are injected with blood, and many polynuclear leucocytes are to be seen in them and migrating through their walls.

Two other monkeys, killed 7 and 9 days after inoculation, showed small areas of pneumonia in their lungs. In both cases the process was characterized by proliferation of the epithelial cells lining the alveoli. No cytoryctes were demonstrable in these lesions.

The exanthem which occurred in this series of monkeys was examined histologically and found to be similar in all respects to that which developed in the other variolated monkeys.

*Summary*.—Four animals were allowed to survive long enough to show an exanthem. Six were killed at various times after the inoculation in order to inspect the initial lesion and to obtain material for histological study. In each of the former animals an exanthem appeared; in 1 on the eighth day, in 2 on the ninth day, and in 1 on the tenth day. The exanthem was of moderate extent and showed an evolution intermediate between that in the monkeys inoculated through a tracheotomy wound and the average case of *variola inoculata* following variolation of the skin.

The constitutional reaction was marked. The temperature reaction was definite, consisting of a distinct rise simultaneously with or immediately before the appearance of the exanthem.

The histological examination of tissues from these animals shows that a lesion similar to that which follows variolation of the mucous membrane of the nose, the lip, and the palate can be produced by inoculation of the epithelium of the trachea. In one animal a variolous bronchitis

and a variolous pneumonia was demonstrable. No focal lesions were found in the bone marrow or testicle.

A series of five Java monkeys (*M. nemistrinus*) was inoculated in the same manner and with the same virus. The results of these experiments were unsatisfactory. No exanthem or constitutional reaction was observed and we were unable to demonstrate a specific lesion at the site of inoculation.

(c) *Inoculation of the lung by inhalation of a spray of the contents of the variola vesicle.*—These experiments were performed to approximate the conditions under which natural infection with smallpox in man might be supposed to occur. Of course, the dose of virus is much larger than it possibly could be under natural conditions, but at least this method approaches more nearly epidemic conditions in that no deliberate trauma to an epithelial surface precedes the distribution or application of the contagium. Five animals were employed in this series, of which the following is selected to be described in detail:

No. 207. The monkey's mouth was held open and a fine spray of vesicle contents (virus No. 199) was thrown into the throat from an atomizer. The animal was observed to breathe while the spray was acting.

No distinct temperature reaction was noted during the 17 days during which the animal was kept under observation. No lesion was observed to develop on the visible mucous membranes. On the seventh day of the disease a papule appeared on the inner aspect of the right arm, at the bend of the elbow. This lesion increased in size, became vesicular, and on the tenth day was distinctly umbilicated. The contents of this lesion was used to inoculate a fresh monkey upon the skin of the abdomen. A typical primary lesion developed and was followed by a profuse general exanthem.

*Summary.*—One of the animals of this series was killed 5 days after inoculation. Inspection of the mucous membrane showed no evidence of a specific lesion. Sections from various parts of the lungs and the trachea were studied microscopically, but no lesions were found. The 4 animals remaining were under observation for 16 days. In one of them a single eruptive lesion appeared on the seventh day of the experiment. This lesion was shown to be specific by inoculation of its contents upon the skin of a fresh monkey.

No constitutional reaction or rise of temperature was observed in this series of monkeys.

(d) *Inoculation of the lung by inhalation of dry variola virus.*—These experiments were devised to determine if *variola vera* could be produced in the monkey by employing the contagious material in a dry condition. Two series of experiments were performed, in one dried pustule contents was used and in the other a powder prepared from desiccated crusts or disks of a case of *variola vera*.

A preliminary experiment was performed to determine how far a powder blown into the larynx would enter the lung. A large monkey was anesthetized with chloroform and a glass tube containing a mixture

of lycopodium and methylene blue powder was introduced into the larynx. During an inspiration the powder was forcibly blown into the lungs. The animal was killed at once and, on dissection, the smallest bronchi which could be made out with the naked eye were found to be stained distinctly blue. Histological examination of the lung showed lycopodium spores in the bronchi and the alveoli.

No. 160. A monkey was put under chloroform anesthesia and the same procedure was followed as above described, except that dry pustule contents was substituted for the methylene blue. The animal was kept under observation for sixteen days. The monkey had a cough from the fifth to the eighth day after the inoculation. On the eighth day of the experiment the body temperature rose to 40°.5 C., and an abundant general exanthem appeared. The lesions of the eruption passed through an evolution which closely simulated that seen in the lesions of a discrete *variola vera* in man. On the sixteenth day of the experiment the animal was vaccinated on the abdomen with virus No. 1. No reaction followed.

Two other monkeys were inoculated in the lung in the same manner and with the same virus. One of these developed symptoms like that above described. The third animal showed a distinct rise of temperature on the third day of the experiment and was immune to subsequent vaccination but did not develop an exanthem. The same method of inoculation was followed in another series of 5 monkeys, but powdered variola disk was substituted for the mixture of pustule contents and lycopodium.

*Summary.*—Two of the animals in the series inoculated with dried pustule contents showed an exanthem. The exanthem appeared on the eighth day of the disease and was profuse. In one of these animals the evolution of the eruption was similar to that seen in *variola vera* in man. A cough was noted in the 2 monkeys which developed an exanthem. The constitutional reaction was not marked, but in each animal a distinct rise in body temperature was observed. The fever began on the eighth day in two and on the sixth day in one.

The monkeys inoculated by inhalation of pulverized variola disks showed no exanthem, no constitutional reaction, and an indefinite temperature reaction. One animal was found to be refractory to a subsequent skin inoculation with variola virus.

(e) *Attempts to inoculate the monkey by exposure to smallpox fomites.*—In the series of inoculations previously described we employed material the infectiousness of which was demonstrable. In all these experiments, although they approximate somewhat the conditions in which man contracts smallpox, the amount of contagious material employed was excessive. In the series to be described we attempted to place the animals under conditions which experience has shown would result in an attack of smallpox if man was subjected to the test, although we were unable to demonstrate the presence of a contagium.

No. 243. Adult male, *M. cynomolgus*. This monkey, together with 4 others, was kept in a cage in which a blanket was placed which had been wrapped around a smallpox patient. The blanket had been in contact with the patient for 6 hours



and was carried from the ward to the animal room in a light-proof, sterile bag. The blanket was left in the cage with the monkeys over night. Observations were recorded upon the monkeys daily for a period of 16 days, during which time no eruption and no temperature reaction appeared. The animal was variolated on the skin on the seventeenth day of the experiment with virus No. 260. A typical primary lesion developed at the site of inoculation.

One of the remaining animals of this series died on the fifteenth day of the experiment, before the immunity had been tested by skin inoculation. At autopsy there was no evidence of variola in this animal. The 3 other monkeys were shown to be susceptible to variolation by skin inoculation.

*Summary.*—Five monkeys showed no clinical symptoms of variola after exposure to a smallpox-infected blanket. Four of these animals were subsequently shown to be susceptible to variola by skin inoculation. The fifth animal died before its immunity was tested, but no anatomical evidence was found that it had contracted variola.

(f) *Attempts to inoculate the monkey by exposure to a smallpox patient.*—This experiment was undertaken as a further test of the reaction of the monkey to conditions which would bring about an infection with smallpox in man.

No. 240. Adult male, *M. cynomologus*. This monkey and 4 others were placed in a cage which was kept in a room for 16 hours with a smallpox patient in the vesicular stage of the disease. The animals were kept under observation for 16 days. No eruption and no temperature reaction was observed. On the seventeenth day of the experiment the animal was inoculated on the abdomen with variola virus No. 260. A typical primary lesion developed at the site of inoculation.

Of the 4 remaining monkeys of this series 2 reacted positively to subsequent variolation on the skin while 2 gave no reaction.

*Summary.*—Five monkeys exposed to a smallpox patient did not develop symptoms of variola. Three of these animals were subsequently shown to be susceptible to variolation by skin inoculation.

(g) *Attempt to inoculate by exposure of the orang-utan to smallpox fomites.*—This experiment was planned to determine if an animal higher in the scale than the monkey would contract smallpox under conditions in which man becomes infected.

No. 197. A young female orang-utan was given a blanket which had just been taken from a case of smallpox in the vesicular stage of the disease. The animal at once wrapped herself in the blanket and used it until the following day, when a clean blanket was substituted for the infected one. During the following eighteen days the animal was kept under constant observation. No exanthem appeared and the variations in the body temperature which occurred were explained by intercurrent infection. The animal was subsequently variolated on the skin and yielded a positive reaction.

*Summary.*—An orang-utan exposed to smallpox fomites did not develop symptoms of variola. The animal was shown subsequently to be susceptible to *variola inoculata* by skin inoculation.

## DISCUSSION.

The experiments detailed above were devised to produce *variola vera* in the monkey or in the orang-utan. An analysis of the results of these experiments shows that of 29 monkeys 10 developed a distinct group of symptoms. Seven of the remaining animals showed no symptoms and 12 were affected in an indefinite way. All the positive results were obtained where some product of the cutaneous lesion of human smallpox was introduced into the animal. All of the negative results were in experiments where smallpox fomites or the air of a smallpox ward was depended upon to carry the contagium. Fifteen experiments in which vesicle contents or pustule contents were either inoculated upon the tracheal mucous membrane or blown into the lung yielded 10 positive results. The disease produced in these animals was characterized by the development of an exanthem, some degree of constitutional reaction, and fever. The evolution of the exanthem was usually similar to that seen in *variola inoculata*, but in 3 animals it resembled the eruption in a mild *variola vera*. The exanthem appeared between the seventh and tenth days of the experiment. The constitutional reaction and the fever appeared on the sixth, seventh, or eighth day of the experiment.

The disease which occurred in these animals agrees with that type which follows variolation of the skin of the monkey, and we have no difficulty in recognizing it as *variola inoculata*.

The negative results which followed exposure of the monkey and the orang-utan to smallpox fomites and smallpox patients show that these animals do not develop a recognizable form of variola when placed under conditions which we believe would produce smallpox in man. We are unable to exclude the possibility of the occurrence of *variola vera sine exanthem* or *variola inoculata sine exanthem* in these animals. The 2 monkeys which were refractory to variolation after exposure to a smallpox patient might owe their immunity to such unrecognizable forms of variola, but we are inclined to regard this phenomenon as very likely due to an individual peculiarity of the animals (natural immunity).

The histological study of the tissues from these series of monkeys brings out certain points of interest. The epithelium of the trachea is shown to be capable of harboring the parasite of the disease, and we see that a lesion can develop in this location which has features in common with the ones produced by variolation of other mucous membranes.

The occurrence of a variolous lesion in the bronchus and, associated with it, a pneumonia in which the parasite is present shows that the organism is capable of multiplying in the deeper parts of the respiratory tract. This fact bears upon the pathogenesis of *variola vera* in man. It is quite conceivable that such a lesion might run its course unnoticed and serve as a focus for the multiplication of the organism during the incubation period of the disease. We have shown that *Cytoryctes variolæ* is capable of infecting the endothelial cells of capillaries and

lymphatics. It is easy to understand that the organisms in a focus of variolous pneumonia might invade the adjacent blood vessels, infect endothelial cells, and so be carried throughout the vascular system. If such an infected endothelial cell were to lodge in a capillary adjacent to an epithelium hospitable to the organism, an eruptive lesion would result. The focal lesions in the seminal vesicles of one of the monkeys which was inoculated in the trachea is doubtless due to such a process, although in this case the infected endothelial cells probably came from vessels beneath the tracheal inoculation. In *variola inoculata* the tissue at the site of the inoculation is flooded with organisms at the time of inoculation, and it seems probable to us that the exanthem developing in these animals results from endothelial cells infected at this time. In *variola vera* the exanthem appears four or five days later than in *variola inoculata*. We believe this interval to be associated with the development of a focus of variolous pneumonia, during which the organisms multiply and finally invade the vessels.

#### CONCLUSIONS.

(1) Inoculation of the mucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus produces a *variola inoculata* in that animal.

(2) Inhalation of variola virus by the monkey (*M. cynomologus*) produces a *variola inoculata* in that animal.

(3) Exposure of the monkey (*M. cynomologus*) and the orang-utan (*Simia satyrus*) to smallpox fomites and to a smallpox patient does not produce *variola vera* or any other recognizable form of variola in these animals.

(4) Inoculation of the mucous membrane of the trachea of the monkey (*M. cynomologus*) with variola virus is followed by the development of a variolous lesion on the mucous membrane which is similar to that produced on other mucous membranes by similar inoculations. A variolous lesion may develop in the bronchi and be associated with a pneumonia in which *Cytoryctes variolæ* are present. The development of the specific lesion in the trachea may be followed by a general cutaneous exanthem and also by focal lesions of a variolous nature in the seminal vesicles.

(5) *Cytoryctes variolæ* can invade the epithelial cells of the trachea, the bronchi, the alveoli of the lung, and the seminal vesicles.

(6) *Cytoryctes variolæ* can invade the endothelial cells of lymphatics and blood vessels. This property of the organism plays an important part in the production of the exanthem in variola.

### PART III.

## STUDIES UPON THE IMMUNITY REACTIONS OF THE MONKEY AFTER INOCULATION WITH VACCINE OR WITH VARIOLA VIRUS.

### 1. ON THE IMMUNITY REACTIONS OF THE MONKEY (*MACACUS CYNOMOLOGUS*) AFTER INOCULATION OF THE SKIN WITH VACCINE OR WITH VARIOLA VIRUS.

In this section we propose to bring together certain observations upon the immunity following the development of vaccinal or variolous lesions upon the skin of the monkey. The data bear upon the general problems of immunity to vaccinia and variola, and we will show certain differences in the immunity reactions of the monkey to the two sorts of virus which throw light upon the general question of the interrelationships of the two diseases. The experiments which form the basis of this section were in part those detailed in other papers of this series and in part the ones performed with special reference to the problems here treated.

The technique used for inoculation was that described in previous papers of this series. The diagnosis of the results of the second inoculations was based upon the descriptions already given of the specific lesions of vaccinia and *variola inoculata* in the monkey. We have been guided wholly by the appearances to the naked eye. In our experience we have rarely been in doubt as to the specific character of a vaccinal or variolous skin lesion in the monkey. In the few instances where we could not feel certain of the diagnosis, the experiment has been ruled out.

### EXPERIMENTS.

1. *Vaccination of the skin after successful vaccination of the skin.*—Thirteen monkeys were selected for this experiment. Each animal had shown a typical vaccinal lesion of the skin of the abdomen from inoculation either with virus No. 1, 148, 236, 246, or 251. Twenty-two days after the occurrence of this lesion the animals were vaccinated on the skin of the abdomen with virus No. 148. All these attempted revaccinations resulted negatively. At the site of the second inoculation there was only the usual, slight reaction which follows a scratch. The scratches healed as if no virus had been used.

2. *Variolation of the skin after successful vaccination of the skin.*—Six monkeys which had shown typical lesions on the skin of the abdomen as

the result of inoculation either with vaccine virus No. 1, 251, 236, 246, or 148 were selected for this experiment. Each animal was variolated on the skin of the abdomen with virus No. 52 or 200. No reaction followed the second inoculation. An interval of from 38 to 58 days elapsed between the two inoculations.

3. *Vaccination of the skin after variolation of the skin.*—The results obtained in this series are best shown by presenting a number of the experiments in detail.

No. 114. Adult male, *M. cynomologus*. Variolated on the skin of the abdomen with virus No. 167 (vesicle contents). Animal developed a typical *variola inoculata* including a general exanthem. On the thirty-seventh day after the variolous inoculation the monkey was vaccinated on the skin of the abdomen with virus No. 148. At the site of inoculation there developed a lesion which had all of the characteristics of a vaccine process only differing from primary vaccinations in the extent of the process and in the indefiniteness of the vesiculation. The lesions passed through a definite evolution and healed spontaneously. We had no hesitation in diagnosing the lesions as specific but typical reactions.

The experiment was repeated on 2 other monkeys of the same species with identical results, which need not be given in detail.

No. 153. Adult male, *M. cynomologus*. The monkey was variolated in a number of places on the skin of the abdomen with virus No. 167 (vesicle contents). A typical, primary lesion developed, which, however, was not so extensive as in the other animals inoculated at the same time with the same virus. No exanthem was noted.

On the tenth day of the experiment the animal was vaccinated on the skin of the abdomen with virus No. 148. An atypical reaction resulted, similar to those seen in the monkeys described above.

On the twenty-fifth day the monkey was vaccinated on the skin of the abdomen with virus No. 1. An atypical lesion was again produced.

No. 141. Adult male, *M. cynomologus*. Variolated on skin of abdomen with virus No. 167 (vesicle contents). *Variola inoculata*, with exanthem, developed. Twenty-eight days after the variolation the animal was vaccinated on the abdomen with virus No. 1. No reaction followed the vaccination.

Two other monkeys were shown to be immune to vaccination of the skin with virus No. 1, 19 days after a variolation on the abdomen with virus No. 167 (pustule contents dried with lycopodium), which had been followed by a typical primary lesion but no general exanthem.

4. *Variolation of the skin after variola inoculata.*—This experiment was only undertaken in one instance and demonstrated the monkey to be immune, after a *variola inoculata*, to a second skin inoculation with variola virus.

#### SUMMARY.

(1) Vaccination of the skin in 13 monkeys protected against subsequent vaccination of the skin.

(2) A vaccination of the skin in 6 monkeys protected against subsequent variolation of the skin.

(3) A variolation of the skin in 3 monkeys protected against subsequent vaccination of the skin. In the case of 3 monkeys the following vaccination yielded a positive, though an abortive, reaction. Another

monkey showed an abortive reaction with 2 successive vaccinations which were subsequent to a variolation.

(4) Variolation of the skin in 1 monkey protected against a subsequent variolation of the skin.

(5) The time which elapsed between the first and second inoculation in these monkeys varied between 10 and 58 days.

(6) Three of the monkeys which were shown to be susceptible to vaccination after successful variolation were tested 37 days after the first inoculation. The 3 animals of the same series which were refractory to the vaccination after *variola inoculata* were tested 28 days after the primary inoculation. The animal which did not seem to acquire an immunity to inoculation (No. 153) was tested on the tenth and twenty-fifth days—that is, previous to the date in which complete immunity was shown to exist in 3 monkeys and to the date in which 3 were shown not to be immune to a second inoculation.

#### DISCUSSION.

The results of our inoculations conform to the general law that vaccination and variolation confer an immunity to subsequent infection with vaccine and variola virus upon the affected animal. The results of similar inoculations in man, which were performed in the early days of vaccination, seem to have yielded more constant results than we have obtained in monkeys. The immunity conferred by a vaccine lesion of the skin of the monkeys is complete against later inoculation with vaccine and variola virus.

The conclusions are not so definite in primary variolation. In a certain proportion of the animals a complete immunity to vaccination on the skin has been produced by a previous *variola inoculata*, but an equal number show only a diminished susceptibility to the vaccine virus. These observations agree with those made by Roger and Weil on *Macacus* monkeys, in which substantially the same phenomenon was noted.

In seeking for an explanation of this partial immunity conferred by *variola inoculata* against vaccination of the skin we might refer it simply to a dying out of the immunity, for we find the completely immune animals were reinoculated on the twenty-eighth day of the experiment, while the animals showing partial immunity were tested on the thirty-seventh day; but this is contradicted by the single animal which was shown to react by a specific process to 3 successive inoculations, with an interval of 10 and 15 days, with variola and vaccine virus (experiment No. 153).

The explanation that there is a qualitative difference in the reaction of this species of animal to the 2 viruses is not borne out by experimental inoculation. The inoculation of variola virus affords partial protection in a certain percentage of cases, and absolute protection in others, to subsequent vaccination.

A third possibility lies in a hypothetical quantitative difference in the immune substance called forth by the 2 sorts of virus.

It seems evident that the immunity which the animal presents to the skin inoculation must depend upon certain properties of the individual conferred upon it by the disease which follows the first inoculation. The weight of evidence is in favor of the immunity being due to a bactericidal or germicidal property resident in the blood serum (Sternberg and Reed, Bécélère, Chambon, and Menard). If such be the case the animals in which complete immunity to vaccinia follows variolation, and in which complete immunity to variolation follows vaccination, indicate that the immune property of the serum of the inoculated animal, whether vaccination or variolation be practiced, is identical. We then would expect to find simply a quantitative difference in this germicidal property of the sera of the animals, depending upon the character of the virus used for inoculation. The reason that *variola inoculata* always protects against an inoculation with variola virus, if confirmed by more experimental evidence than we present, would be that the variola virus is less potent upon the monkey than vaccinia virus, in that it fails to develop in monkeys protected by previous variolation, whereas the latter develops and produces a lesion.

That the immunity resulting from inoculation of the monkey with variola virus is less efficient than that resulting from vaccination is apparent from the fact that vaccination protects against both subsequent variolation and vaccination, while variolation protects against subsequent vaccination and only partially against subsequent vaccination.

At the present time technical difficulties prevent the putting of the quantitative aspects of the above hypotheses to the test of experiment.

#### CONCLUSIONS.

(1) A vaccine lesion on the skin of the monkey (*M. cynomologus*) confers upon the animal an immunity to subsequent inoculation of the skin with vaccine or with variola virus.

(2) A variolous lesion on the skin of the monkey (*M. cynomologus*) protects the animal against subsequent inoculation of the skin with variola virus, but does not in all cases protect against later inoculation with vaccine virus.

(3) The failure of *variola inoculata* in the monkey to protect against subsequent skin inoculation with vaccine virus depends upon the fact that this species of animal produces a smaller amount of the germicidal substance necessary to inhibit a second inoculation after variolation than it does after vaccination.

#### 2. ON THE INFLUENCE OF THE LOCUS OF INOCULATION UPON THE DEVELOPMENT OF THE IMMUNITY IN VARIOLA AND VACCINIA IN THE MONKEY (*MACACUS CYNOMOLOGUS*).

*Introduction.*—In the preceding section we have studied the immunity reactions of the monkey to inoculation of the skin with variola and with vaccinia virus and have brought out certain differences in the immunity

produced by the two viruses. In this section we will detail experiments which bear upon the general problem of the immunity reactions of the monkey to vaccine and to variola virus from a somewhat different point of view.

In testing the immunity of rabbits after skin and after corneal inoculations with vaccine virus, Dr. R. L. Thompson<sup>7</sup> obtained results which tended to show a difference in the degree of immunity depending on the *locus* chosen for the primary vaccination. As we were more favorably situated as regards facilities and animals for experimentation, we decided to continue this line of work on monkeys. We have extended the scope of the experiments so as to include the study of the relative immunity produced by vaccination and variolation of the skin, cornea, and mucous membranes.

#### EXPERIMENTS.

(a) *Vaccination of the cornea after vaccination of the skin.*—This experiment was performed upon 5 monkeys (*M. cynomolgus*). Each animal had shown a typical vaccinal lesion on the skin as a result of an inoculation with virus No. 1, 148, 236, or 251, and each was tested 22 days after the first inoculation by vaccination of the skin with virus No. 148. The cornea was vaccinated with virus No. 148 on the twenty-ninth day after the initial skin vaccination. The animals were killed after 48 hours, and paraffin sections of the corneas were examined microscopically.

Four of the monkeys showed no specific lesion on the cornea. One showed a typical vaccinal keratitis with proliferation of the epithelium and the presence of numerous cytocytes.

(b) *Variolation of the cornea after variola inoculata from skin inoculation.*—This series consisted of 5 monkeys which had shown a typical primary lesion on the abdomen following inoculation with variola virus No. 167 (disk). Three of these animals had developed a general exanthem.

On the twenty-fourth day of the experiment each monkey was inoculated on the cornea with variola virus No. 167 (vesicle contents). The animals were killed after 72 hours and the corneas studied microscopically. Each animal presented a typical variolous keratitis at the site of inoculation, and cytocytes were present in large numbers.

(c) *Variolation of the skin after variolous keratitis.*—This experiment was performed on a single animal. The cornea was inoculated with variola virus No. 200. A typical lesion developed. Eighteen days after the corneal variolation variola virus No. 252 was inoculated on the skin of the abdomen without producing a lesion. The skin inoculation was repeated on the forty-first day with variola virus No. 307, and again no reaction followed.

(d) *Vaccination of the skin after variolation of the mucous membrane of the palate.*—The 5 monkeys employed for this experiment had developed a typical variolous lesion on the soft palate, following variolation with virus No. 167 (vesicle contents). Each animal was vaccinated on the skin with virus No. 148.

<sup>7</sup>These experiments were carried on in the pathological laboratory of the Boston City Hospital under the direction of Dr. W. T. Councilman. Owing to the impossibility, at the time, of carrying out the research on extensive enough lines to yield definite conclusions, the results were not published.



on the twenty-first day of the experiment. In every animal a typical vaccine lesion developed at the site of inoculation.

(e) *Variolation of the skin after variolation of the mucous membrane of the palate.*—Three monkeys were selected that had shown a typical variolous lesion on the soft palate after inoculation with virus No. 307. Eighteen days after the initial inoculation the animals were variolated on the abdomen with virus No. 307. Two of the monkeys showed no reaction to the skin inoculation, while 1 yielded a typical primary lesion, but no exanthem developed.

(f) *Vaccination of the skin after variolation of the mucous membrane of the lip and nose.*—One monkey was inoculated in this way. Typical variolous lesions developed on the inner side of the lip and on the nasal septum after inoculation with variola virus No. 167 (vesicle contents). The monkey was vaccinated on the abdomen with virus No. 148 and No. 1 on the seventeenth and forty-first days, respectively. No lesion developed after either vaccination.

(g) *Vaccination on the skin of the temple after vaccination of the abdomen.*—Two monkeys that had shown a typical vaccine lesion on the abdomen were later shown to be refractory to vaccination on the temple.

#### SUMMARY.

(a) A vaccine lesion of the skin protects against subsequent inoculation of the cornea with vaccine virus, but the protection is not complete.

(b) A variola lesion of the skin does not protect against subsequent inoculation of the cornea with variola virus.

(c) In one monkey a variola lesion on the cornea protected against subsequent inoculation of the skin with variola virus.

(d) A variola lesion on the mucous membrane of the palate does not protect against subsequent inoculation of the skin with vaccine virus.

(e) A variola lesion on the mucous membrane of the palate does not completely protect against subsequent inoculation of the skin with variola virus.

(f) A variola lesion on the mucous membrane of the lip and nose protected, in one instance, against subsequent inoculation of the skin with vaccine virus.

(g) A vaccine lesion on the skin of the abdomen protected against subsequent inoculation of the skin of the temple with vaccine virus.

#### DISCUSSION.

The summary of our experiments in this section demonstrates that the immunity produced by a variolation on the mucous membrane is lower than that produced by a variolation on the skin.

We have already shown that the immunity produced by a variolation on the skin is lower than that following a vaccination on the skin, and we find that this is emphasized by the results of inoculations of the cornea after vaccination and variolation of the skin. The fact that even vaccination of the skin does not completely protect against subsequent corneal inoculation with vaccine virus illustrates our point with regard to the quantitative relation of the two immunities.

In interpreting the results of these inoculations the following factors must be considered, viz:

- (a) The *locus* of the initial inoculation.
- (b) The virus used in the initial inoculation.
- (c) The *locus* of the second inoculation.
- (d) The virus used in the second inoculation.

Bearing these factors in mind we see that *loci* chosen for initial inoculations bear the following relation to the resulting immunity.

The immunity conferred by skin *locus* is greater than that by the cornea, and the latter is greater than that by the mucous membrane. The immunity conferred by vaccine is greater than that conferred by variola, as we have already indicated in the preceding section.

The influence of the *locus* of the second inoculation can only be estimated in regard to the cornea and skin when we find that the immunity conditioned by the initial inoculation is less efficacious when the cornea is chosen than when the skin is the site of the second inoculation.

The influence of the sort of virus used in the second inoculation upon the test seems to indicate that the vaccine virus is more potent than the variola virus in that it may produce a lesion in an animal in which there is complete protection to inoculation with variola.

We find, in short, that the skin is relatively a more efficacious *locus* than the cornea, and the latter is more efficacious than the mucous membrane in producing immunity. The immunizing power of vaccine virus is higher than that of variola virus.

If we interpret the above experiments in the light of the hypothesis elaborated in the previous section and keep in mind the physical conditions at the various *loci* of inoculation, we feel that the phenomena observed are quite consistent.

A variolation of the cornea after skin variolation succeeds because the total amount of immune substance present in the individual is relatively small, owing to the character of the virus used in the initial inoculation, and because the physical conditions on the cornea do not favor a free mixing of the immune-bearing plasma with the inoculated virus.

In the case of a monkey vaccinated on the skin a vaccination of the cornea only rarely succeeds, because the relatively large amount of immune substance present, even under the adverse physical conditions in the cornea, usually is sufficient to produce a germicidal effect upon the inoculated virus. We feel that the fact of an occasional animal yielding a positive reaction to such a second inoculation only emphasizes our view that the phenomenon is a quantitative one.

In the case of the mucous membrane as a *locus* of initial inoculation the physical conditions are doubtless a large factor in causing the low immunizing power. Study of the lesion on the mucous membrane shows that almost from the first an open wound is present at the site of inoculation. This condition would favor the discharge of toxine and products

of degeneration of the organism, and would be unfavorable for the production of an immune substance which resulted from the reaction of the host cells.

The high potentiality of the skin as a *locus* of the initial inoculation for immunity production is in sharp contrast with that of the mucous membrane, and the physical conditions are in keeping with the interpretation given above. In a skin lesion the greater part of the products of the lesion must be absorbed and go to produce the general immunity.

It seems probable from the histological study of the specific lesion in the nose that this *locus* would stand nearer to the skin in its potentiality for immunity production than the lip or palate. We feel that our data are insufficient for generalization on this point.

#### CONCLUSIONS.

(1) The degree of protection conferred by a vaccinal or variolous lesion on the monkey (*M. cynomologus*) is conditioned by the *locus* chosen for inoculation as well as by the virus which is employed.

(2) The varying degree of immunity production which follows the development of vaccinal or variolous lesions at different *loci* of inoculation is dependent upon the physical conditions there present.

(3) The outcome of an inoculation of an animal which has had a variolous or vaccinia lesion depends upon the *locus* and upon the virus employed in the second inoculation as well as upon the *locus* and upon the virus employed in the first one.

#### 3. ON THE TIME OF DEVELOPMENT OF THE IMMUNITY AFTER INOCULATION OF THE SKIN OF THE MONKEY WITH VACCINE AND WITH VARIOLA VIRUS.

*Introduction.*—The following experiments were planned to show the interval which elapses between the inoculation of the monkey's skin with vaccine or variola virus and the development of an immunity inhibiting further inoculations. The results of these experiments bear upon the general problem of the diseases, and particularly upon that of the causation of the exanthem in *variola inoculata*. The experiments are arranged in three series, as follows:

(a) *Daily inoculation of the skin with vaccine virus.*—Five monkeys (*M. cynomologus*) were selected and each received upon the skin of the abdomen a single vaccination daily for 9 days. The development of each lesion was observed and objective descriptions recorded from day to day. Vaccine virus No. 148 was employed.

No. 108. The lesions from vaccinations performed on the second, third, fifth, sixth, and seventh days of the experiment showed a typical development. Those from inoculations on the first and fourth days were abortive in character, vesiculation not being complete. The inoculations done on the eighth and ninth days were entirely negative.

No. 109. The first, second, third, fourth, and sixth vaccinations were positive, while no reaction followed those on the fifth, seventh, eighth, and ninth days of the experiment.

No. 110. The first to the fifth vaccinations, inclusive, resulted positively, while those on the 4 succeeding days were not followed by a reaction.

No. 111. The first to the fifth vaccinations, inclusive, yielded a typical vaccine process, while the remaining 4 were without result.

No. 112. The first, second, and third vaccinations were negative, the fourth was abortive, and the fifth, sixth, seventh, eighth and ninth were negative.

(b) *Daily inoculation of the skin with variola virus.*—Eight monkeys (*M. cynomologus*) were used in this series of experiments. Each animal daily received a single inoculation with variola virus. Variola virus No. 167 (vesicle contents), No. 199, or No. 200 was employed, and after the final inoculation the sample was tested by inoculation upon the skin of a fresh monkey and shown still to be potent.

No. 136. The inoculations on the first, second, third, fourth, and fifth days of the experiment yielded typical lesions. The fifth, sixth, and seventh inoculations were not followed by a lesion. A general exanthem developed on the ninth day of the experiment.

No. 137. The first to the fourth inoculations, inclusive, were positive, while the remainder were negative. An exanthem appeared on the ninth day.

No. 138. The first 4 inoculations were positive, which the last 3 were negative. An exanthem developed on the eighth and ninth days.

No. 213. The first, second, third, and fourth inoculations were positive, while the fifth, sixth, and seventh were negative. On the seventh, eighth, and ninth days a general exanthem was observed.

No. 214. The inoculations on the second and third days yielded positive, those on the first and fourth abortive, and those on the fifth, sixth, and seventh negative reactions. No general exanthem developed.

No. 215. The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. No exanthem developed.

No. 116. The first, second and third inoculations were positive, the fourth was questionable, the fifth, sixth, and seventh were negative. A general exanthem appeared on the eighth and ninth days.

No. 117. The first four inoculations were positive, the fifth was questionable, the sixth and seventh were negative. An exanthem appeared on the ninth day.

(c) *Simultaneous inoculation with vaccine and variola virus.*—Five monkeys were selected and inoculated on the left groin with vaccine virus No. 1, and on the right side of the chest with variola virus No. 200.

Four animals reacted typically to both inoculations and 2 of these developed an exanthem on the ninth day of the experiment. The fifth monkey reacted typically to the vaccine virus, but did not show a process at the site of the inoculation with variola virus or develop an exanthem.

## SUMMARY.

(1) In 2 monkeys daily inoculations with vaccine virus ceased to produce a positive lesion 5 days after the first inoculation.

(2) Three monkeys similarly vaccinated failed to react 6 days after the first successful inoculation.

(3) In 4 monkeys inoculated daily with variola virus positive reactions were not obtained 4 days after the first inoculation.

(4) In 2 monkeys inoculated daily with variola virus, an abortive lesion followed the inoculation done 4 days after the first one, and in 2 animals a similar lesion developed from an inoculation performed 3 days after the primary inoculation.

(5) Six of the 8 monkeys subjected to daily inoculations with variola virus developed an exanthem. The eruption was first present on the ninth day in 2, on the eighth day in 3, and on the seventh day of the experiment in 1.

(6) The interval between the last successful daily inoculation (counting abortive lesions as positive) and the appearance of the exanthem was 5 days in 2 monkeys, 4 days in 3, and 3 days in 1.

(7) Four monkeys reacted to both vaccine and variola virus, simultaneously inoculated, by the appearance of typical lesions which developed apparently without influencing one another. Two of these animals developed a general exanthem on the ninth day of the experiment.

## DISCUSSION.

A comparison of the results of daily inoculation of the skin with vaccine virus with those following similar inoculation with variola virus shows that there is a distinct difference in the time of onset of the immunity.

In the experiments where vaccine was used the refractoriness to skin inoculation, if judged by the day on which the first unsuccessful inoculation was performed, appeared, on an average, during the seventh day of the experiment—that is to say, the seventh daily inoculation, which was performed 6 days after the first successful one, fails to show a specific reaction.

In the series where variola virus was employed this refractoriness to reinoculation appeared, on an average, during the fifth day of the experiment.

Without committing ourselves to the exact date of onset of the immunity, we may yet assert from this that it is of earlier development in *variola inoculata* than in *vaccinia*. The determination of the exact day of development of an immunity to subsequent skin inoculation can not accurately be determined by this procedure. In studying the evolution of a vaccine or a variola lesion on the skin, we see that an interval of from 72 to 96 hours intervenes between the inoculation and the appearance of a process which can be diagnosed by the naked eye.

That this incubation period is apparent rather than real, as shown by a microscopic study of sections from the inoculation sites, does not help us in this connection. By the methods adopted in these experiments we are in doubt for 3 days as to the outcome of our inoculation. At any time during this period the development of the lesion may be checked by the onset of the immunity. We then see that the fact that the inoculations on the first 4 days of an experiment result in a diagnosable lesion, while those on the fifth day and on succeeding days do not develop to a diagnosable condition, does not fix the date of onset of immunity at the fifth day. Obviously an inoculation done on the fifth day might not appear in the records of the experiment as a positive reaction if the immunity developed even 2 days later, as the lesion would be inhibited before it was recognizable.

Therefore we must conclude that the onset of the immunity is not before the date of the last successful inoculation, and may be as much as 3 days later. Applying this conclusion to our experiments, where daily inoculation was practiced, we see that the development of immunity to reinoculation of the skin with vaccine virus may manifest itself at any time between the sixth and the eleventh days, and to reinoculation with variola virus between the fifth and the eighth days. These figures are arrived at by selecting the days of the earliest unsuccessful inoculation for the earliest date and the day of the latest unsuccessful inoculation plus three for the latest date. These limits, while wide, are as narrow as we believe to be warranted by the method of experimentation adopted.

The appearance of a general exanthem in the animals inoculated daily with variola virus, from 3 to 5 days after the last successful insertion of virus on the skin, seems at first sight difficult to explain. If we allow for a 3-day interval between the invasion of the skin and the appearance of the eruptive lesion, we see that this brings the date of invasion, and hence the period of intravascular transit of the organisms to within the limits set for the onset of the immunity. The number of organisms which go to produce a regular, eruptive lesion are undoubtedly infinitely fewer than those introduced in an inoculation of the skin with virus. As the growth of the lesion depends upon the multiplication of the organisms, it is probable that the interval between the invasion of the skin and the appearance of the exanthem lesion is longer than that between inoculation of the skin with virus and the diagnosable stage of the primary lesion. We must conceive of the organisms which are to produce the exanthem as passing from the primary lesion to the skin before the date of onset of the immunity. By comparing the dates we can readily understand how the organisms might make this intravascular journey before the immunity developed. Another explanation lies in the possibility that phagocytes act as carriers and as protectors of the organism from the immune plasma. The development of an exanthem is therefore quite consistent with our conclusions with regard to the time of onset of

the immunity. The brief evolution and abortive development of the lesion of the exanthem is what might be expected in an animal which had already developed such a germicidal power in its plasma that its presence in the inoculation wound, and in the subsequent exudate stream of inflammatory origin, inactivated the virus introduced at the site of a skin inoculation.

In the lesion developing spontaneously on the skin, the immune plasma doubtless does not have as free access to the organisms as is the case where the virus is mixed in a scratch with fresh drawn blood serum.

The phenomenon of an exanthem in *variola inoculata* and its absence in vaccinia is not explained by these experiments. Had it proved that the general immunity was of notably later development in *variola inoculata* the exanthem producing quality of variola virus would readily have been explained. However, the reverse seems to be the case, and we have to seek further for the explanation of this fundamental difference between the two viruses.

Simultaneous variolation and vaccination of the monkey shows that the synchronous development of a vaccine lesion has no effect upon the appearance of the exanthem of *variola inoculata*. The fact that vaccination on or about the date of exposure to smallpox inhibits the production of clinical types of *variola vera*, characterized by an exanthem, emphasizes the difference between the diseases *variola vera* of man and *variola inoculata* of monkeys.

In a previous section we have shown that the immunity potential of the mucous membrane is low. In *variola vera* it seems exceedingly probable that the atrium of infection and the site of the primary lesion is on a mucous membrane. If such be the case we would expect that little if any immunity would develop as a result of the evolution of this lesion, and the organisms which seek the skin to produce the exanthem would develop in a practically nonimmunized animal. This agrees with the course of the exanthem in a typical *variola vera* in man. In other cases of smallpox the exanthem shows an evolution very like that in *variola inoculata* (*variola abortiva*), or is absent (*variola sine exanthem*), and in these we conceive the organisms which go to form the exanthem as acting against a more or less fully developed immunity. This condition might be dependent upon an early development of the general immunity arising from the primary pock, and be conditioned by its *locus*. In any case, a vaccination on the skin at the time of exposure produces an immunity which develops before the exanthem, probably killing the organisms in transit from the protopustule to the skin, and so inhibiting the eruption.

This suggests an explanation of the failure of all attempts to abort the exanthem in *variola vera* by the injection of what certainly were highly germicidal sera (Béclère and others).

It is evident that at the time when the case is fully declared to be smallpox and is put under treatment, the organisms are inaccessible

to the serum. To be effective the serum would have to be given before the disease had advanced to a diagnosable state. Such sera might be useful in cases where a patient well advanced in smallpox is discovered in an unvaccinated family. The unprotected ones in contact with such a case will probably be in the incubation stage of smallpox, and might be protected by injections of serum from a *variola vera* with exanthem.

#### CONCLUSIONS.

(1) The immunity which accompanies the development of a vaccine lesion on the skin of a monkey becomes manifest between the sixth and eleventh days.

(2) After a variola lesion of the skin the immunity appears between the fifth and eighth days.

(3) The organisms which produce this exanthem in *variola inoculata* in the monkey pass from the point of inoculation to the skin before the onset of the general immunity.

(4) The development of an exanthem in *variola inoculata* in the monkey is not dependent upon a late development of the immunity reaction of the animal.

(5) The use of variolical sera is indicated only in cases where it can be administered during the incubation stage of the disease.



#### PART IV.

### ON THE OCCURRENCE OF CYTORYCTES VARIOLÆ GUARNIERI IN EXPERIMENTAL VARIOLA AND VACCINIA IN THE MONKEY AND IN THE ORANG-UTAN.

In a previous publication from the Department of Pathology of the Harvard Medical School, the specific inclusions found in the cells in vaccinia and variola, which have been the subject of investigation by Guarnieri and others,<sup>8</sup> were described at length, and the conclusion was reached that they were intracellular organisms. In addition to the bodies in the protoplasm, which are common to the lesions of vaccinia and of variola, a series was found within the nuclei of the epithelial cells in the specific lesions of variola. These intranuclear bodies were first described by Councilman, Magrath, and Brinckerhoff, and were regarded by them as phases of the organism peculiar to smallpox.

In the present paper the name "Cytoryctes" will be applied to the specific nuclear as well as to the specific cytoplasmic forms. Since the morphology of these various forms has fully been described by a number of observers,<sup>9</sup> this phase of the problem will not be treated in this paper, attention being directed to the occurrence and distribution of these inclusions in various lesions produced experimentally in monkeys and in the orang-utan.

*Literature.*—Since the preliminary report on variola by Councilman, Magrath, and Brinckerhoff,<sup>10</sup> Bosc has published a series of articles relating to smallpox and vaccinia. He describes in vaccinia and in variola cytoplasmic forms which possess definite structure and which pass through a developmental cycle resulting in multiplication. In variola he describes an additional cycle within the nucleus, and the forms pictured are similar to those discovered by Councilman. He believes these structures to be the causal agent in the disease.

Ewing (7) considers the cytoplasmic forms of cytoryctes as products of degeneration of the cytotreticulum, in support of which he cites the intimate relation of the bodies to the reticulum of the cell. The cycle presented by the various forms he believes to be one of degeneration

<sup>8</sup> For literature to 1904 see *Journal of Medical Research*, 11, 116.

<sup>9</sup> Councilman, Magrath, and Brinckerhoff (3), Calkins (1), Howard and Perkins (9), Bosc (4) (5).

<sup>10</sup> Councilman, Magrath, and Brinckerhoff (2), May, 1903.

rather than development, and is unable to distinguish certain features, such as nuclear structure, spore formation, and multiplication, which characterize the well-known protozoan parasites.

Ewing finds the nuclear forms specific to smallpox, but does not consider that sufficient proof has been adduced to identify them as organisms. He finds them identical in staining reaction with nucleoli and linin globules and claims to demonstrate transitions between the latter and the larger vacuolated forms. The presence of these structures in cells advanced in degeneration he regards as against the parasitic theory.

Howard and Perkins (9) confirm the life cycle of cytoryctes as presented by Calkins and present a hitherto undescribed secondary cytoplasmic stage. They believe that it is this form which first invades the nucleus and gives rise to the nuclear phases previously described.

Ewing (8) in a more recent publication presents results obtained by the application of the *Klatsch* method of making histological preparations. He claims that by this method there is less artefact, especially as regards shrinkage, than in tissue fixed and sectioned by the usual methods. After applying this technique to the study of vaccine lesions of the cornea and skin he concludes that the vaccine body is a portion of the cytotreticulum, the alteration in its staining reaction being due to a diffusion of nuclear proteids into the cytoplasm. He suggests the possibility of the presence of specific organisms within these "degenerations." He admits the vaccine bodies to be specific to variola and vaccinia.

Siegel (11) confirms previous descriptions of the cytoplasmic forms, but suggests that the nuclear forms described by Councilman are post-mortem artefacts. He bases this inference on the statement of Tyzzer that he was unable to find these intranuclear forms in perfectly fresh tissue, and utterly disregards the experimental work on monkeys in which the presence of the nuclear bodies was shown in perfectly fresh tissue.

Davidson (6) studied the tissues of a case of variola and found the cytoplasmic but no nuclear forms.

Shrumpf (10) does not consider either the nuclear or cytoplasmic inclusions to be organisms. His conclusions do not seem to be based upon very extensive studies.

*Technique.*—The details of inoculation in the series of experiments from which our material for this study was obtained are given in full in other sections of this paper and need not be repeated. However, attention is called to the fact that the method of making multiple inoculations upon the skin furnished lesions of sufficient number to excise one at each 24-hour interval from the time of inoculation until repair had begun. By this method it is possible to obtain all stages of the process in each animal. In the study of the disease produced in other ways than by inoculation upon the skin, animals of a given series were killed at regular

intervals, usually at 24-hour periods, from the time of the inoculation and the lesions and tissues fixed for histological study. All tissue was at once put in Zenker's fluid for fixation and afterwards embedded in paraffin by the alcohol-chloroform method. Sections having a thickness of about 5 microns were cut, except in a few instances when sections of one-half this thickness were prepared. The Mallory eosin-methylene-blue method of staining gives very satisfactory results for the study of cytoryctes and was generally used. A large number of the staining methods now in common use were tried, but none was found to be specific for cytoryctes.

The lesions upon which this histological study is based were obtained chiefly from Philippine monkeys (*M. cynomologus*) inoculated with vaccine or with variola virus. These were supplemented by variola lesions of the Java monkey (*M. nemestrinus*), variola lesions of the orang-utan (*Simia satyrus*), and primary vaccinations from the human subject. The material furnished by the various series of experiments is as follows:

1. *Vaccine lesions of the skin*.—Fifteen Philippine monkeys each with multiple vaccinations: A lesion excised at each 24-hour interval from 1 to 10 days after inoculation. Eleven other monkeys killed at various periods after vaccination. Two vaccine lesions of the human subject obtained 5 and 7 days, respectively, after vaccination.

2. *Vaccination of the cornea*.—Nine monkeys furnishing lesions of 17, 24, and 48 hours, and 3, 4, 5, 7, and 8 days' duration.

3. *Vaccinations of mucous membrane*.—Nine monkeys vaccinated upon the inner surface of the lip, on the nasal septum, and on the soft palate. Killed at 24-hour intervals from 1 to 9 days.

4. *Primary variola lesions of the skin*.—Ten monkeys each receiving multiple inoculation: A lesion excised each day as in the skin vaccination series. Eleven other Philippine monkeys killed at various intervals after inoculation. Three Java monkeys: Lesions excised at 24-hour intervals from 3 to 9 days after variolation. An orang-utan furnished lesions 6, 7, and 8 days after variolation.

5. *Variolations of the cornea*.—Nineteen monkeys.

6. *Primary variola lesions of the mucous membrane*.—Nine monkeys variolated on the inner surface of the lip, on the nasal septum, and on the soft palate: Killed at 24-hour intervals from 1 to 9 days. Five monkeys variolated on the nasal septum alone: Killed 2, 3, 4, 5, and 6 days after the inoculation.

7. *Primary variola lesions of the trachea*.—Eight monkeys. In one of these there was also a specific process in the lung, possibly an extension from the trachea.

8. *Variola exanthem*.—Lesions appearing at various locations upon the skin after an interval of 6 to 10 days after inoculation with variola. The eruption of one case in which it was profuse upon the mucous membrane of the oral cavity and oesophagus as well as upon the skin. In this case there were also lesions of a specific nature in the seminal vesicle which were considered to be a part of the eruption.

This material furnishes data on the occurrence of cytoryctes in lesions produced by the inoculation of three species of apes with variola, and in vaccine lesions of the Philippine monkey and of man.

## THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN VACCINIA.

Cytoplasmic forms are found in every lesion in which there is a characteristic process following the inoculation of vaccine. In the vaccination lesions of the skin, cytoryctes are usually demonstrable 48 hours after inoculation and in a few instances 24 hours after inoculation. They persist in the lesions up to 8 days after the vaccination and may occasionally be found in small numbers for a longer period. They are found at the sides of the vesicles where there is a gradual transition from normal epithelium to that in which degeneration is advanced. The small, deeply stained, and sharply defined forms are found at the periphery where the epithelium is nearly normal. The expanded indefinite forms which Calkins has described as amoeboid forms, and residual masses from which the granules have disappeared are found in older portions of the lesions. These indefinite forms are present in lesions of 72 hours and in some cases in those of 48 hours duration. In the early lesions they are found in relatively small foci, but as the process advances they are distributed through a greater mass of the epithelium. They are often distinguishable in cells advanced in degeneration, but are not so in the fluid of the vesicle. In several lesions cytoryctes were found in the endothelium and in other cells about a small superficial blood vessel, just beneath the vesicle.

The process produced by the vaccination of the cornea is accompanied in every instance by the cytoplasmic forms of cytoryctes. With the extensive destruction of epithelium in these inoculations, a considerable surface is denuded so that cytoryctes are present only in small numbers at the edge of the degenerating epithelium.

In the vaccine lesions of the lip, nose, and soft palate cytoryctes are constantly present. Their distribution is similar in these lesions to that of the skin lesions, the small, sharply defined forms occurring at the periphery and the larger expanded forms in the older portion of the process.

In this large number of vaccine lesions produced by the inoculation of monkeys with 4 different strains of vaccine virus, the nuclear forms of cytoryctes as found in variola do not occur. However, in vaccine lesions it is not uncommon to find nuclei distended with eosin-stained material in the form of reticulum, hyaline granules or globules. In some cases inclusions of this sort are abundant. The eosin-stained masses may have a regular, definite contour, or they may be faint and indefinite in outline. The reticular masses usually have an irregular or frayed periphery. Greatly distended nuclei may contain a large number of rounded globules of uniform size, which in some instances show a tendency to vacuolation, or in others may possess deeply staining centers. These inclusions often contain masses of deeply stained material, evidently the chromatin of the epithelial cells. However, vaccine lesions of the skin, the cornea, and the mucous membrane of the lip, nose, and soft

palate, taken at all stages of the process from its beginning up to the time of repair, fail to show the presence of the specific nuclear bodies found in variola. Two primary vaccine lesions from a native child, obtained at 5 and 7 days, respectively, after vaccination, present no nuclear forms such as are found in variola lesions.

#### THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN PRIMARY VARIOLA LESIONS.

Primary lesions produced by the inoculation of Philippine monkeys (*M. cynomologus*) with variola virus are constantly associated with the cytoplasmic forms. The distribution of these forms in variola skin lesions differs in one respect from their distribution in vaccine lesions of the skin. In the process which is present beneath the epithelium in the primary variolation, and which involves the dermis and subcutaneous tissue, cytoryctes are present in large numbers. They are found with endothelial cells *in situ* within the vessels or lying in the adjacent tissue. The endothelium of blood vessels as well as lymphatics is thus affected. Three Java monkeys (*M. nemestrinus*) afforded skin variola lesions of a peculiar type. The process affects but a small area of the epithelium, while the dermis and subcutaneous tissue show an extensive lesion. Cytoplasmic forms are present in the endothelial cells found in this region, also in sections in which they are not demonstrable in the epidermis.

The time occurrence of the cytoplasmic forms is practically identical with that in vaccine lesions of the skin. They are present in lesions of 48 hours, and in some instances in those of 24 hours' duration. They usually disappear from the lesion 9 days after inoculation, but occasionally they are found subsequent to this in small foci. Their distribution in the epithelial portion of the lesion is similar to that in vaccine lesions. The nuclear forms of cytoryctes are found in the majority of lesions resulting from variolation of the skin of both the Philippine and the Java monkey. In no case are these forms numerous. In many the study of a single section through the lesion reveals one of several of these forms. A prolonged search through a great many sections is necessary in other cases to find a single typical nuclear form. They are usually found in cells forming the floor of the vesicle or in those of the hair follicles where the degeneration of cells is advanced. Associated with the typical nuclear forms are other inclusions which, however, are not specific to variola. These appear as masses of stringy reticulum, hyaline bodies, granules, and globules within the nuclei, similar to the forms already described in vaccinia. They may include portions of the nuclear chromatin and may all be duplicated in vaccine lesions, but are not nearly so abundant there as in the variola lesion. The reaction of these structures to the eosin-methylene blue stain is apparently identical with that of the specific nuclear forms.

In the skin variolations, hyaline masses within the nuclei may show a tendency to vacuolation or they may have definite rings embedded in

their substance. Intranuclear forms of cytorcytes were found in variolations of the skin from 3 to 8 days after inoculation. As they were so uncertain in their occurrence one could not well judge at what time they were most numerous. In all cases they were preceded by the cytoplasmic forms and were never found without these.

In primary variola lesions of the orang-utan taken 6, 7, and 8 days after inoculation, nuclear forms are very abundant. A variety of both the specific forms and the nonspecific reticular and hyaline bodies may be found in a single section. In this respect the skin variolations in the orang-utan differ greatly from those of the two species of monkey employed, which show relatively few nuclear forms. Cytoplasmic forms are present in these variola lesions of the orang-utan. Their distribution is similar to that in the preceding species.

In the lesions following variolation of the monkey's cornea the cytoplasmic forms are constantly present but never the nuclear forms. The cytoplasmic forms appear rather more numerous than in vaccinations of the cornea, probably on account of the retention of the epithelium. They are found in lesions of 24 hours after inoculation and are present in those of 11 days' duration, beyond which time we have no material. This non-occurrence of intranuclear forms is probably due to physical conditions.

In the primary inoculations of the lip, the nose, and the palate the occurrence and distribution of both cytoplasmic and nuclear forms are essentially the same as those found in skin variolations. In one instance the nuclei of the nose lesion were filled with the nonspecific eosin-staining inclusions, but there were but few typical intranuclear bodies.

In lesions of the trachea produced by inoculation with variola virus cytoplasmic forms are present. In one case a specific variolous pneumonia accompanied the inoculation of the trachea and cytorcytes were present within the epithelial cells lining the alveoli. In this case the process appears to have extended from the bronchus into the lung substance.

#### THE OCCURRENCE OF CYTORCYTES VARIOLE IN EXANTHEM LESIONS.

A general eruption followed the inoculation of various sites upon the body with variola virus. Eruptions were produced by the inoculation of the skin, the cornea, the mucous membrane of the lip, nose, and palate, the trachea, and by intravenous injection of variola virus. The lesions of the eruption appear after periods of from 6 to 10 days subsequent to inoculation.

Cytoplasmic forms are found constantly in these eruption lesions, excepting in those in which repair is advanced. Except in the exanthem resulting from intravenous injection, the specific nuclear forms are rare. However, they are found in the exanthem following skin inoculation in several cases, and it is probable that by a study of a greater number of lesions and a longer series of sections they would be shown to occur in a large number of cases. The exanthem following intravenous

injection furnishes a greater number of nuclear forms than any other experimental lesion, with the exception of the primary lesion of *variola inoculata* in the orang-utan; in this respect it approaches the character of the *variola vera* eruption in man.

Besides the lesions appearing as an eruption on the surface of the body following inoculation with variola virus, similar ones may appear on the mucous membranes, or in more remote organs. Following the inoculation of the tracheal epithelium of a monkey through an incision in the neck, there was not only a profuse eruption upon the skin, but likewise upon the mucous membrane of the mouth, cheek pouches, and œsophagus, and also in the seminal vesicles. In all these lesions the cytoplasmic forms of cytoryctes are present.

#### THE OCCURRENCE OF CYTORYCTES VARIOLÆ IN REINOCULATIONS.

In many animals which had been inoculated upon the skin with variola virus, a subsequent inoculation of the cornea with variola or vaccine virus resulted in a typical reaction. The corneal lesions in these cases contain cytoryctes which can not be distinguished from those of the lesions of first inoculations.

Lesions produced by the inoculation of dried smallpox or vaccine virus appeared later and ran a milder course than was the case with fresh virus. In these lesions cytoryctes were present in small numbers, this apparently being due to the limited area affected. They were morphologically identical with those occurring in other lesions.

#### SUMMARY AND CONCLUSIONS.

(1) The cytoplasmic forms of *Cytoryctes variolæ* are found constantly in all specific lesions resulting from inoculation with variola or with vaccine virus. They appear in the primary lesions of both *variola inoculata* and vaccinia soon after the inoculation. They persist in the primary skin lesions for about 8 days after inoculation, at which time immunity is established and repair is beginning. In *variola inoculata* the exanthem as well as the primary lesions contain cytoplasmic forms.

(2) Intracellular forms are found within the epithelial nuclei in lesions resulting from the inoculation of the monkey with variola virus and do not occur in vaccine lesions. These structures are specific for variola. Other nonspecific, nuclear inclusions occur in vaccinia, in variola, and in other nonrelated processes.

(3) The nuclear forms of cytoryctes, which are found only in small numbers in the primary skin lesion of *variola inoculata* in the monkeys (*M. cynomologus* and *M. nemestrinus*), are present in far greater numbers in the corresponding lesion of the orang-utan.

(4) Nuclear forms were only occasionally found in lesions of the general eruption following the inoculation of the skin of the monkey with variola virus, but were very numerous in the eruption which followed the intravenous injection of variola virus into the tail vein,

with subsequent amputation of the tail proximal to the point of inoculation. This eruption resembles in this, as well as in other respects, the eruption of *variola vera* in man.

(5) The cytoplasmic forms of cytoryctes are constantly associated with variola and vaccinia in whatever portion of the body the lesions may develop. Thus they are found included in a variety of cells, the squamous epithelium of the skin, the cornea, the mucous membrane of the nose, oral cavity, and oesophagus, the cells of the sebaceous and Meibomian glands, the epithelium of the conjunctiva, the columnar epithelium of the nose, trachea, and seminal vesicle, the epithelium lining the alveoli of the lung, endothelial cells, and connective-tissue cells.

(6) The occurrence of cytoryctes in the cells of the corium, and especially within the endothelium of vessels, suggests a possible method of dissemination of the organism in the production of the exanthem. However, endothelial cells containing cytoryctes were also found in a few instances in vaccine lesions, a form of the disease never accompanied by a general eruption.

(7) The occurrence and distribution of the specific inclusions is best explained by the hypothesis that they are parasites, and that as such they are the cause of the disease.

#### REFERENCES.

- (1) CALKINS, G. N., 1904. The life history of *Cytoryctes variolæ* Guarnieri. *Journal of Medical Research* (1904), XI, 136.
- (2) COUNCILMAN, MAGRATH, and BRINCKERHOFF, 1903. A preliminary communication on the etiology of variola. *Journal of Medical Research* (1904), XI, 372.
- (3) COUNCILMAN, MAGRATH, and BRINCKERHOFF, 1904. The pathological anatomy and histology of variola. *Journal of Medical Research* (1904), XI, 12.
- (4) BOSC, F. J. Le parasite de la vaccine. *Compt. rend. Soc. Biol.* (1903), LV, 1178.
- (5) BOSC, F. J. Le parasite de la variola. *Compt. rend. Soc. Biol.* (1903), LV, 1204.
- (6) DAVIDSON, C. Tödlieh verlaufender Pockenfall. *Berliner klin. Wochensch.* (1905), XLV, 635.
- (7) EWING, J., 1904. Comparative histology of Vaccinia and Variola. *Journal of Medical Research* (1904), XII, 511.
- (8) EWING, J., 1905. The structure of vaccine bodies in isolated cells. *Journal of Medical Research* (1905), XIII, 233.
- (9) HOWARD, W. T., and PERKINS, R. G. A study of the etiology of variola. *Journal of Medical Research* (1904), XII, 360.
- (10) MAGRATH and BRINCKERHOFF, 1904. On the occurrence of *Cytoryctes variolæ*, Guarnieri, in the skin of the monkey inoculated with variola virus. *Journal of Medical Research* (1904), XI, 173.
- (11) SCHRUMPF, P. Ueber die als Protozoen beschriebenen Zellenschlüsse bei Variola. *Virchow's Arch.* (1905), CLXXIX, 461.
- (12) STEGEL, J. Untersuchungen über die Aetiologie der Pocken und der Maul- und Klauenseuche. *Verlag der Königl. Akademie der Wissenschaften, Berlin* (1905).



## PART V.

### ON THE REACTION OF VARIOLA VIRUS TO CERTAIN EXTERNAL CONDITIONS.

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*Introduction.*—In the experiments described in the other parts of this report we have been engaged with the reactions of the inoculated animal to the contagium of smallpox and of vaccinia. In this section we propose to emphasize the changes which the contagium itself undergoes under certain experimental conditions.

The contagium of variola is demonstrable in the specific skin lesions of human smallpox and in the lesions produced in various animals by inoculation with such material. The proof of the presence of the contagium depends upon the results of the inoculation of a suitable animal with the suspected material, as no cultural or micro-chemical technique has been devised which permits of the certain identification of the organism, save in sections of the specific lesion. This being the case, we have only limited criteria for estimating the quality of a given sample of virus. By inoculation of the monkey we can determine whether or not the virus will produce the typical variolous lesion, and if it does so, we can classify the lesions produced by different strains of virus according to the course of development of the primary lesion, the occurrence and extent of the exanthem, and the degree of constitutional reaction. From these data we may draw certain inferences as to the quality of the samples of virus tested. We give below the results of such tests of various samples of virus which had been treated in such wise as to modify their properties.

(a) *The effect of keeping upon variola virus.*—No. 71. A sample of virus collected from a case of smallpox, at autopsy, in the pustular stage, produced a positive reaction when inoculated on the abdomen of a monkey (*M. cynomologus*). The virus was placed in an ice box where it was exposed to a varying temperature, which at times reached many degrees above freezing. Fifty-seven days later the virus was found to have a putrid odor. Inoculations on the abdomen of a monkey and on the cornea of a rabbit yielded no reaction of any sort.

Another sample of virus became putrid after a few days keeping, yet yielded typical takes when inoculated on the monkey's skin. On many occasions vesicle contents, sealed in capillary tubes and kept on ice, was found potent after three weeks.

No. 44. Variola disks collected from cases of the epidemic in Boston during February, 1902, were tested sixteen months later. The material had been subjected to the high temperature of the steamer's hold during the journey across the Pacific. Inoculations on the monkey's skin and on the rabbit's cornea were negative.

No 48. Vesicle contents collected from a case in the Philadelphia epidemic during January, 1902, sealed in capillary tubes and exposed to the same conditions as the Boston disks, was found inactive when inoculated on the rabbit's cornea 4 months later.

(b) *The effect of drying upon variola virus.*—This series of experiments was undertaken to test the resistance of variola virus to drying at room temperature. For comparison, parallel experiments were made with vaccine virus.

The ends of a number of glass rods were coated with a thin film of vesicle contents from a case of smallpox. Similar preparations were made from the five-day vaccine lesions of a calf. The rods were at once put into sterile test tubes, closed with cotton plugs, and kept at room temperature.

After two weeks the rods coated with vaccine virus produced typical lesions on the monkey's skin when rubbed upon a fresh scratch. The rods coated with variola virus were inactive. After three weeks the variola rods were again tested and found inactive. A final test of the vaccine rods three and a half weeks after preparation showed them to have also become inactive.

(c) *The effect of passage through the Berkefeld filter upon variola virus.*—A sample of variola virus (No. 199, vesicle contents) was shown to be active when inoculated upon the skin of monkeys. A portion of the virus was filtered through a small Berkefeld filter, "N". The filtrate was inactive when inoculated on the skin of 2 monkeys and on the cornea of 1 rabbit. The monkeys used in this test were later shown to be susceptible to skin inoculation with variola virus. The unfiltered portion of virus No. 199 was shown to be active for many days after the test of the filtered portion.

(d) *The effect of glycerin upon variola virus.*—In a previous publication<sup>11</sup> we stated that we had found variola virus to be rendered inactive by mixture with glycerin. The following tests were made to control the results of our preliminary experiments along this line:

No. 252. A large amount of variola virus (vesicle contents) was collected from a case of smallpox at autopsy. The material was put on ice and after 24 hours a portion of it was mixed with 60 per cent glycerin in the proportion of 1 part virus to 3 parts glycerin. The mixture was thoroughly shaken and kept on ice. The glycerinated virus was tested from time to time by inoculation on the monkey's skin.

No. 255. Monkey inoculated on the abdomen with the glycerinated virus after 3 days on ice. A typical primary lesion developed, but its evolution was somewhat delayed. An exanthem consisting of 2 typical lesions appeared on the tenth day.

No. 273. Monkey inoculated on the abdomen with the glycerinated virus after 11 days on ice. A typical primary lesion developed and was followed by an exanthem consisting of 3 small vesicles on the tenth day.

No. 318. Monkey inoculated as before, with glycerinated virus which had been on ice for 26 days. A typical primary lesion developed which was delayed in its evolution and was not followed by an exanthem.

No. 363. Monkey inoculated as before with the glycerinated virus which had been kept on ice for 50 days. The primary lesion was typical but delayed in its evolution. No exanthem developed.

<sup>11</sup> *Jour. Med. Research*, 11, 296,

(e) *The effect upon a strain of variola virus of repeated passages through the monkey.*—No. 200 virus. A series of 5 monkeys was inoculated with this strain of virus, each animal save the first being inoculated with curettings of the primary lesion of the previous monkey of the series. Typical primary lesions developed on each animal. The exanthem was profuse after the first and second serial inoculation, but was sparse in the third and fourth animal. The strain was transferred to 2 additional monkeys from the primary lesions of the second monkey of the series, and in each of these animals an atypical primary lesion developed, but was not followed by an exanthem. After the third serial transfer the strain was inoculated on another monkey, yielding an atypical primary lesion followed by a sparse eruption. A transfer from the primary lesion of this animal resulted in an atypical primary lesion and no exanthem.

A series of 4 monkeys was inoculated by transfer of virus from the exanthem, beginning with the second animal of the first series described. Each animal showed a typical primary lesion save the last, in which the process was somewhat delayed. A profuse exanthem developed in each animal.

The strain was inoculated at each transfer of the above series to another monkey and in each case yielded an atypical primary lesion. The first transfer was followed by a moderately extensive exanthem.

The second animal of the series of inoculations with the virus from the exanthem was used as a source of virus for testing the immunity of certain monkeys. The contents of the primary lesion was employed. Three animals inoculated with this virus developed typical primary lesions, which were followed by a sparse general exanthem.

No. 199 virus. This strain of virus was carried through one orang-utan and two monkeys. The first monkey showed a typical primary lesion and a profuse exanthem. The second monkey did not react to the inoculation.

The time of the occurrence and the evolution of the exanthem in all the monkeys of these experiments was similar to that seen in animals inoculated with fresh human variola virus.

#### SUMMARY.

(1) A variola virus (vesicle contents and disk) lost its power to produce a specific lesion upon the skin of the monkey after prolonged exposure to high temperature, to putrefaction, and to desiccation.

(2) A sample of variola virus lost its power to produce a specific lesion on the monkey's skin and on the rabbit's cornea by passage through the "N" Berkefeld filter.

(3) A sample of variola virus retained its power to produce a primary lesion, but lost its exanthem-producing properties after exposure to 60 per cent glycerin for 50 days.

(4) A strain of variola virus showed less and less power to cause a typical primary lesion on the monkey and a waning exanthem producing potentiality after repeated passages through monkeys.

#### DISCUSSION.

The reaction of vaccine virus to drying and to the action of glycerin has been carefully determined by those who have charge of the preparation of vaccine for protective inoculations against smallpox. It is a matter of common knowledge that vaccine retains its potency for a

comparatively long time in the dry state. Our experiments with variola virus are not very extensive and we hesitate to draw broad conclusions from them, but so far as they go they show that variola virus is less resistant to drying than vaccine. This whole question of the comparative resistance of the two sorts of virus to physical conditions is unsettled and presents an attractive field for study.

In our earlier experiments we found that variola virus did not retain its potency in the presence of 60 per cent glycerin. As the results of our later experiments upon this point show variola virus will resist the action of glycerin for a considerable period, we are inclined to believe that our first experiments were done with a glycerin which was not absolutely neutral, but that the inactivation of the virus was due to an acid in the glycerin and not to the action of the pure reagent. The loss of the power to develop an exanthem after prolonged contact with glycerin is of interest and should be made the basis of further work. It is important to determine whether this loss is associated with any change in the development of the two cycles of the parasite.

The question of the passage of the variola and vaccine contagium through the filter has been the subject of a certain number of experiments. We have only one observation to record upon this, which shows that the organism is held back by the filter. It is to be noted that this inactivation of the virus by filtration was tested by skin inoculations on the monkey and did not exclude the presence of diffusible contagium in the filtrate.

The influence of serial transfers upon the variola virus is shown in our series. This demonstrates that the virus tends to die out when transferred from monkey to monkey. This is in contrast with the stability of a strain of vaccine on an animal.

The question of the relative virulence of different strains of virus requires for its intelligent discussion the criteria for judging of the virulence of a given virus. In the case of variola virus the reactions of the animal inoculated consist in the development of a local lesion, the production of an exanthem, the tumefaction of the lymph nodes, and in some constitutional disturbance. The two former present many degrees of variation and seem most suitable as a basis for judgment as to the virulence of the virus inoculated. We can distinguish between various reactions at the site of inoculation and classify them as typical, atypical, abortive, etc. We can observe the occurrence, the extent, and the evolution of the exanthem. If we had only the virus to consider, we should classify our different strains or samples of virus according to the degree of local reaction at the site of inoculation and the nature of the exanthem. However, such a direct interpretation is impossible, as we must take into consideration the degree of natural immunity of the animal chosen for inoculation to a virus. In order to estimate this element it is necessary to compare the results of the inoculation of

several animals with the same sample of virus. If the majority of the animals yielded atypical or abortive lesions, we should be warranted in saying that the virus was avirulent. If, on the other hand, a majority of the animals gave typical lesions, we should classify the virus as virulent.

Applying this test to all our experiments, we find that certain strains of variola virus we have used may be classified as virulent and others as avirulent. We may also say that the transfer of a strain of variola virus from one monkey to another tends to reduce its virulence. It also seems to be the case that drying, either in the course of the disease, as in the formation of the crust, or artificially, reduces the virulence. In the same way, exposure to 60 per cent glycerin lowers the virulence.

This brings up the question of the nature of the difference between variola and vaccine virus. The two strains of virus may be said to bear a relation of virulence and avirulence to one another, so far as the results of our experiments go. However, there is an important difference between the two contagiums which we have not been able to bring out experimentally but which is a matter of common knowledge. This difference may be put as follows: Vaccine produces a local lesion at the site of inoculation, but no exanthem, and is not air-borne; variola produces a local lesion at the site of inoculation, an exanthem, and is air-borne.

The occurrence of an exanthem and of an air-borne contagium still needs explanation. In the extenuation of a strain of variola virus we see that the exanthem-producing potentiality is lost before the virus becomes entirely inactive. Variola virus which has lost its power to produce an exanthem has practically no points of difference from a vaccine virus, so far as its reactions on the monkey are concerned. Whether or not it has in fact become a vaccine virus could only be determined by inoculations and by exposure experiments with human beings. Such procedures are of course impossible and the solution of the problem must await the findings of an experimental animal in which *variola vera* can be produced under the same conditions which produce the disease in man.

#### CONCLUSIONS.

- (1) Variola virus is less resistant to desiccation than vaccine virus.
- (2) Variola virus does not pass through the "N" Berkefeld filter.
- (3) Variola virus is attenuated by long exposure to 60 per cent glycerin. The virus so treated loses its power to produce an exanthem when inoculated on the skin of the monkey (*M. cynomologus*).
- (4) Variola virus tends to die out when passed repeatedly through the monkey. The exanthem-producing power is lost before the virus has become incapable of producing a primary lesion.

## ILLUSTRATIONS.

### PLATE I.

- FIG. 1. *Variola inoculata* in the orang-utan (*Simia satyrus*). Lesion at the site of inoculation. Duration 6 days. Wide spread vesicle formation extending outward from the inoculation wound.
2. *Variola inoculata* in the orang-utan (*Simia satyrus*). Lesion at the site of inoculation. Duration 7 days. Vesicle contains more fluid than before. Multilocular character of lesion well shown. False vesicle formation to the right of the inoculation wound.

### PLATE II.

- FIG. 1. *Variola inoculata* in the Philippine monkey (*Macacus cynomologus*). Lesion at the site of inoculation. Duration 6 days. Dark mass in center is the crust. On either side there is vesicle formation and at the periphery of the lesion thickening of the epidermis. Marked œdema and cellular infiltration of the corium beneath the lesion.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem in the skin of the palm of the hand. The lesion is well advanced in the process of healing.

### PLATE III.

- FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem. Vesicular stage. Well-marked cellular infiltration about the vessels of the corium.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem. Pustular stage.

### PLATE IV.

- FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion of the exanthem in cheek pouch. Animal was inoculated in the trachea.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Focal lesion in the seminal vesicle, of the nature of an exanthem. From same animal as in fig. 1.

### PLATE V.

- FIG. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion at the site of inoculation in the nose. The process in a stratified epithelium.
2. Same as fig. 1, but inoculation is on a columnar epithelium. Note the cellular infiltration of the submucous tissue.

## PLATE VI.

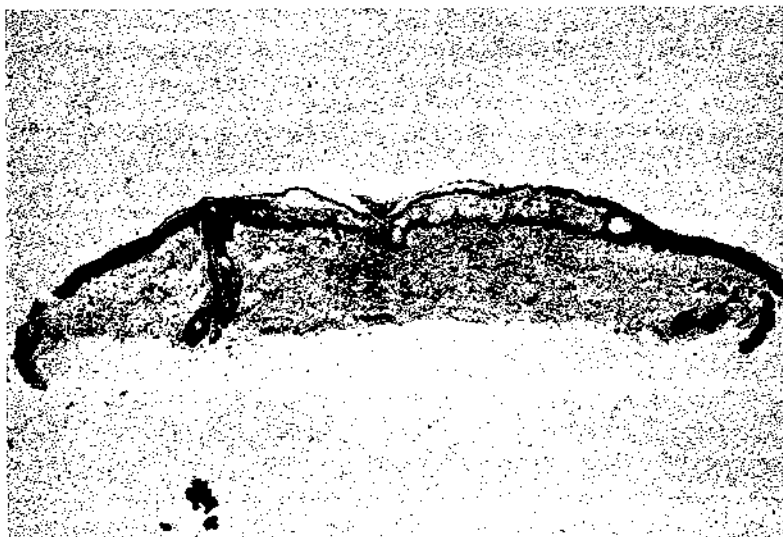
1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Lesion at the site of inoculation on the trachea. Well-marked, tongue-like projections of the epithelium due to swelling and proliferation of the epithelial cells. This stage proceeds one in which the surface becomes denuded of epithelium. Duration 6 days.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Variolous lesions in the bronchus and in the lung following inoculation of the trachea. Variolous bronchitis and variolous pneumonia. Duration 6 days.

## PLATE VII.

- Fig. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic phase of *Cytoryctes variolæ* in the epithelial cells of the alveoli of the lung in a variolous pneumonia.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic forms of *Cytoryctes variolæ* in the epithelial cells of the seminal vesicle.

## PLATE VIII.

- Fig. 1. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). *Cytoryctes variolæ*, protoplasmic forms in the epithelium of the trachea.
2. *Variola inoculata* in the Philippine monkey (*M. cynomologus*). Protoplasmic form of *Cytoryctes variolæ* in an endothelial cell in a capillary beneath a lesion of the palate of 3 days' duration. ( $\times 2000$ .)



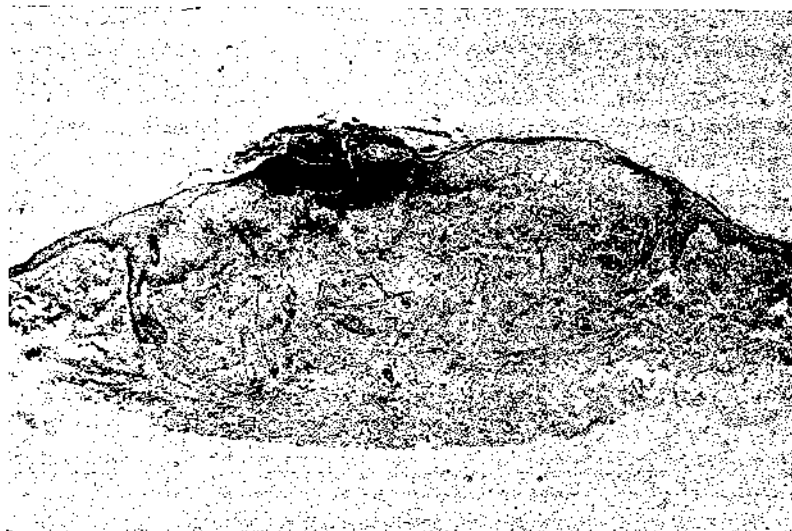
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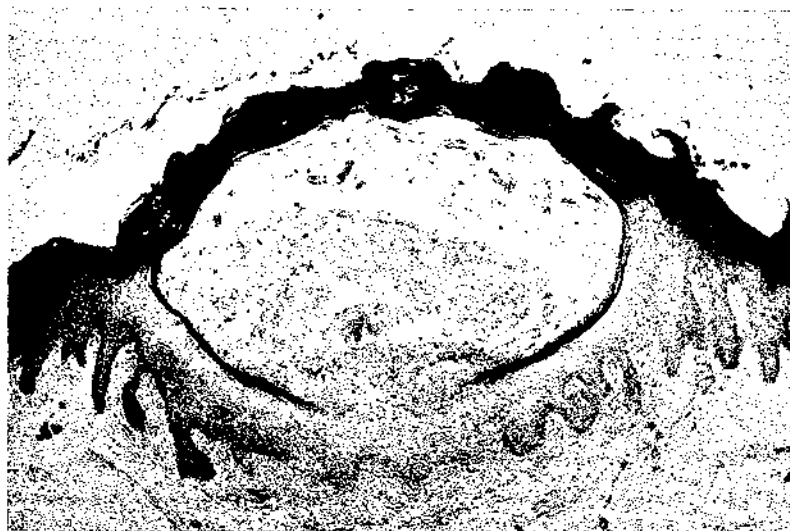
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PLATE I.



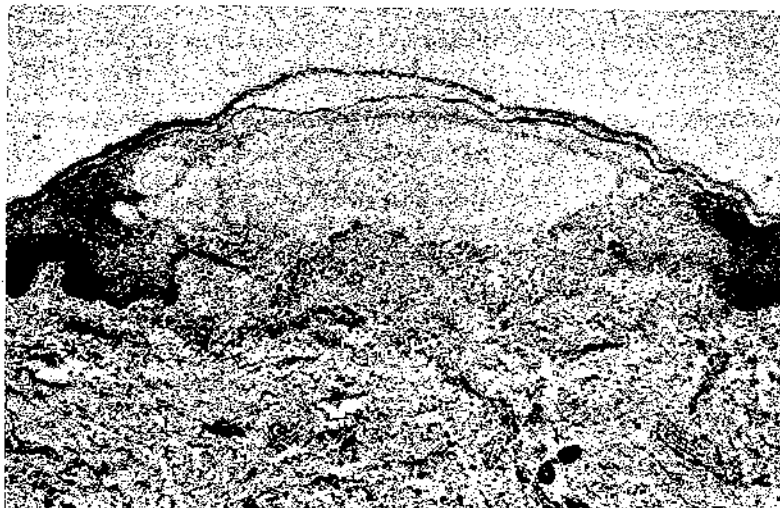


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2.

PLATE II.



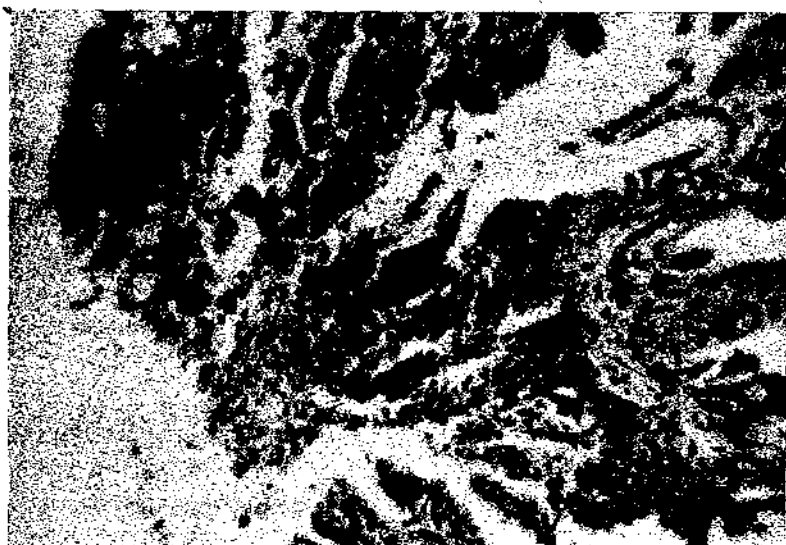
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2.



1.



2.

PLATE IV.



1.

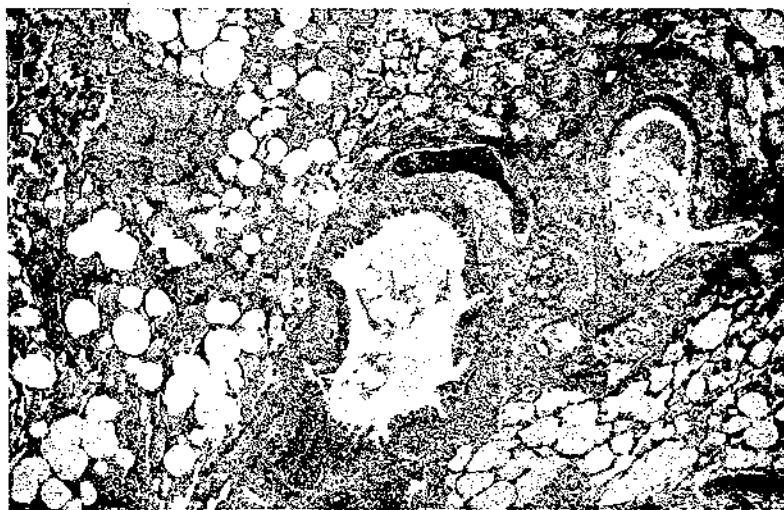


2.

PLATE V.

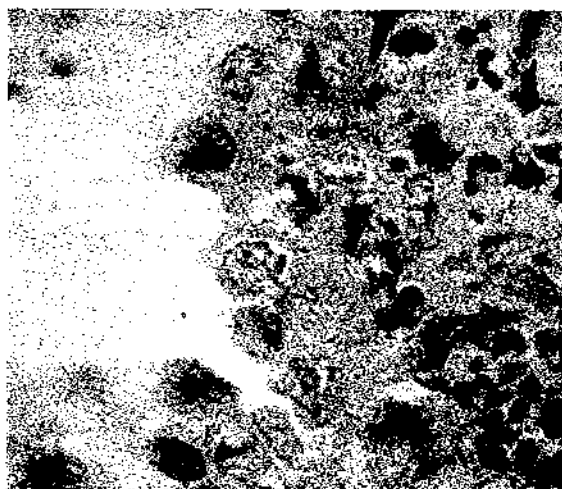


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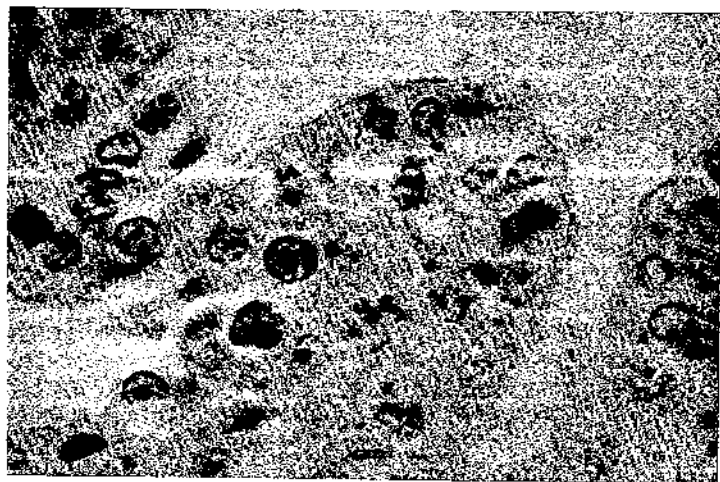


2.

PLATE VI.



1.



2.